BONE MINERAL DENSITY AND BONE TURNOVER MARKERS IN HEALTHY PRE AND POSTMENOPAUSAL WOMEN AND THE INFLUENCE OF MULTIPLE FACTORS ON THEM

Dissertation submitted to the

The Tamil Nadu Dr. MGR Medical University,

Tamil Nadu

in partial fulfillment of the rules and regulations for the degree of

DM Endocrinology examination to be held in August 2015,

Christian Medical College, Vellore

BONE MINERAL DENSITY AND BONE TURNOVER MARKERS IN HEALTHY PRE AND POSTMENOPAUSAL WOMEN AND THE INFLUENCE OF MULTIPLE FACTORS ON THEM

by

Dr. Sahana Shetty

Dissertation submitted to

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY,

In partial fulfillment of the requirements for the degree of DM in ENDOCRINOLOGY

Under the Guidance of

Prof. Thomas V Paul

Co-Guidance of

Prof. Nihal Thomas

Department Of Endocrinology, Diabetes and Metabolism,

Christian Medical College, Vellore, Tamil Nadu

DECLARATION

I hereby declare that this dissertation titled **"Bone Mineral Density and Bone Turnover Markers in Healthy Pre and Postmenopausal Women and the influence of multiple factors on them"** was carried out by me under the direct supervision and guidance of Prof. Thomas V Paul and co-guidance of Prof. Nihal Thomas, Professor and Head, Department of Endocrinology, Diabetes & Metabolism, Christian Medical College, Vellore.

This dissertation is submitted to The Tamil Nadu Dr. MGR Medical University, in partial fulfillment of the requirements for the degree of DM in Endocrinology.

I also declare that this dissertation has not been submitted by me to any other university or for the award of any other degree.

Vellore

Date: Christian Medical College, Vellore

CERTIFICATE

This is to certify that this research work titled **"Bone Mineral Density and Bone Turnover Markers in Healthy Pre and Postmenopausal Women and the influence of multiple factors on them"** was done by Dr. Sahana Shetty, DM in Endocrinology, Christian Medical College, Vellore.

This study was undertaken in the Department of Endocrinology, Diabetes and Metabolism, Christian Medical College, under my direct supervision, guidance and to my complete satisfaction, as a part of the requirement for the award of the degree DM in Endocrinology.

Dr. Thomas V Paul, MBBS, MD(Gen Med), DNB(Endo), PhD(Endo),
Professor & Guide,
Department of Endocrinology, Diabetes & Metabolism,
Christian Medical College, Vellore – 632 004.

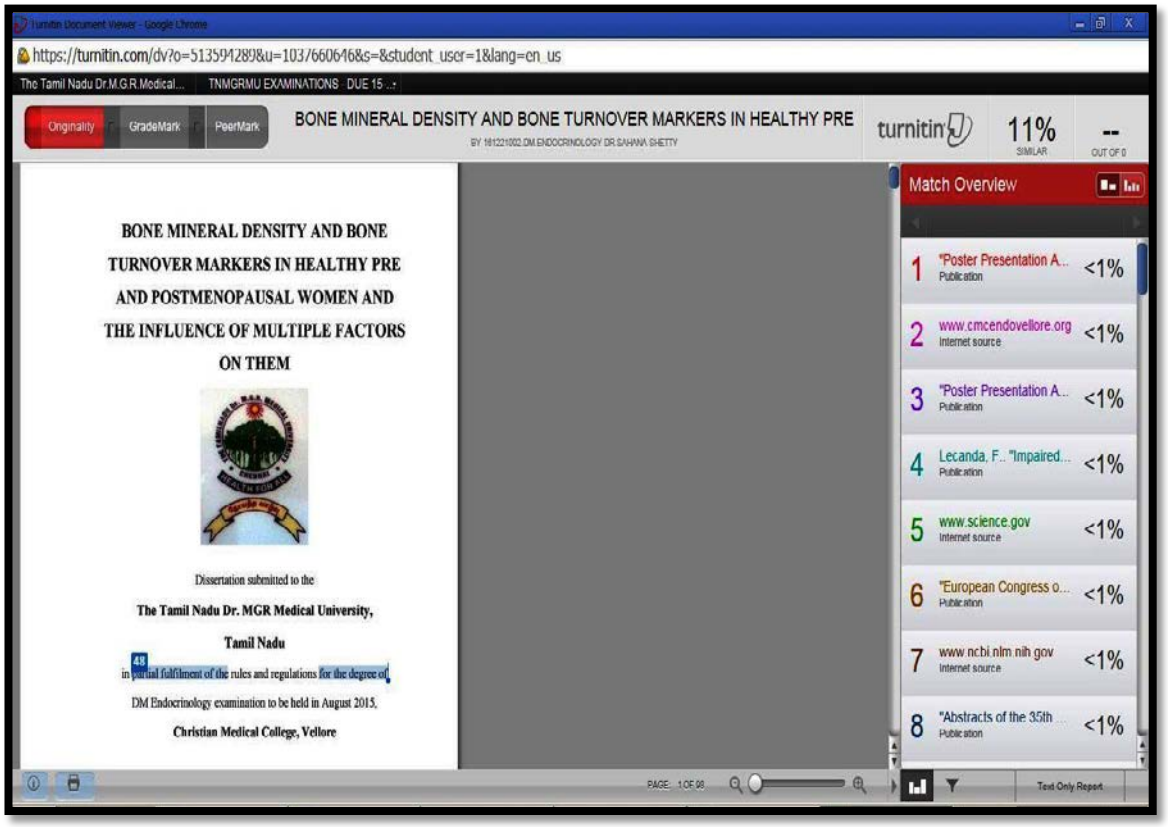
Dr. Nihal Thomas,
MBBS, MD, MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin), FRCP(Glasg),
Professor & Head,
Co-Guide,
Department of Endocrinology, Diabetes & Metabolism,
Christian Medical College, Vellore – 632 004.

CERTIFICATE

This is to certify that this research work titled **"Bone Mineral Density and Bone Turnover Markers in Healthy Pre and Postmenopausal Women and the influence of multiple factors on them"** was done by Dr. Sahana Shetty, DM in Endocrinology, Christian Medical College, under the guidance of Professor Thomas V Paul and co-guidance of Professor Nihal Thomas, Professor and Head, Department of Endocrinology, Diabetes & Metabolism, Christian Medical College, as a part of the requirement for the award of the degree DM in Endocrinology.

Professor Alfred Job Daniel, MBBS,D.Ortho, DNB(Ortho), MS(Ortho),
Principal,
Christian Medical College, Vellore 632 002.

ANTIPLAGARISM CERTIFICATE



OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

June 10th 2014

Dr. Sahana Shetty
Senior PG Registrar
Department of Endocrinology, Diabetes & Metabolism,
Christian Medical College, Vellore 632 004.

Sub: **FLUID Research grant project:**
Bone Mineral Density and bone turn over markers in healthy pre and postmenopausal women and the influence of multiple factors on them.
Dr. Sahana Shetty, Endocrinology, Dr. Thomas V Paul, Dr. Nitin Kapoor, Dr. Nihal Thomas, Dr. Simon Rajaratnam, Dr. Asha HS, Dr. Dhukhabandu Naik, Endocrinology, Diabetes and Metabolism, Dr. Jude Joseph Fleming, Clinical Biochemistry, Dr. J. Visalakshi, Biostatistics.

Ref: IRB Min. No. 8343 [OBSERVE] dated 18.06.2013

Dear Dr. Sahana Shetty,

The Institutional Review Board [Blue, Research and Ethics Committee] of the Christian Medical College, Vellore, reviewed and discussed the study title "Bone Mineral Density and bone turn over markers in healthy pre and postmenopausal women and the influence of multiple factors on them".

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Glas) (EDIN)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on June 10th 2014 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, CMC, Vellore	Internal, Clinician
Dr. Suresh Devasahayam	BE, MS, PhD	Professor of Bio-Engineering, CMC, Vellore	Internal, Basic Medical Scientist
Dr. B. Poonkuzhali	MSC, PhD	Professor, Haematology, CMC, Vellore	Internal, Basic Medical Scientist
Dr. Anuradha Bose	MBBS, DCH, MD,MRCP, FRCPCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Sathya Subramani	Md. PhD	Professor, Physiology, CMC, Vellore	Internal, Clinician
Dr. Anil Kuruvilla	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Deepak Abraham	MBBS, MS	Professor, Endocrine Surgery, CMC, Vellore	Internal, Clinician
Dr. Thambu David	MBBS, MD, DNB	Professor & Head, Medicine, CMC, Vellore	Internal, Clinician
Dr. L Jeyaseelan,	M. Sc, PhD, FRSS	Professor, Biostatistics, CMC, Vellore	Internal, Statistician
Prof. Keith Gomez	BSc, MA (S.W), M. Phil (Psychiatry Social Work)	Student counselor, Loyola College, Chennai, Deputy Chairperson, Ethics Committee, IRB	External, Lay Person & Social Scientist

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Glas) (EDIN)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Mrs. Pattabiraman	B. Sc, DSSA	Social Worker, Vellore	External, Lay person
Mr. C. Sampath	B. Sc, BL	Legal Expert, Vellore	External, Legal Expert
Dr. P. Zachariah	MBBS, PhD	Retired Professor, CMC,	External, Scientist
Dr. Jayaprakash Muliyil	BSC, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, Vellore	External, Scientist & Epidemiologist
Dr. Vinitha Ravindran	PhD (Nursing)	Professor & Addl. Deputy Dean, College of Nursing, CMC, Vellore	Internal, Nurse
Mrs. Ruma Nayak	M.Sc (Nursing)	Professor, Head of Paediatric Nursing & Deputy Nursing Superintendent, College of Nursing, CMC, Vellore	Internal, Nurse
Dr. Binu Susan Mathew	MBBS, MD	Associate Professor, Clinical Pharmacology	Internal, Pharmacologist
Dr. Nihal Thomas	MD, MNAMS, DNB(Endo), FRACP(Endo) FRCP(Edin) FRCP (Glasg)	Professor & Head, Endocrinology. Additional Vice Principal (Research), Deputy Chairperson, IRB, Member Secretary (Ethics Committee), IRB, CMC, Vellore	Internal, Clinician

We approve the above amendment as presented.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Glas) (EDIN)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. Nihal Thomas was not present for the final deliberations and the decision was taken by a neutral body of the IRB.

Yours sincerely

Dr. Alfred Job Daniel
Principal & Chairperson (Research Committee)
Institutional Review Board

NOT TO BE MINISTERED UNTO BUT TO MINISTER
CHRISTIAN MEDICAL COLLEGE
VELLORE
INDIA

CC: Dr. Thomas V Paul, Endocrinology, Diabetes and Metabolism, CMC.

Acknowledgement

Expression of gratitude and thankfulness in words is a rare opportunity one can avail. Penning down this acknowledgement, I take the opportunity to express my gratitude to my mentors, colleagues, family and friends, who helped me accomplish this task that seemed prodigious but ended as a gratifying journey.

Words won't suffice to express my thankfulness to my guide and mentor for this research venture, ***Dr. Thomas V Paul****, Professor, Department of Endocrinology, Christian Medical College, Vellore. I thank him for his exceptionally versatile guidance, valuable suggestions, critical appreciation and keen interest that led to successful compilation of this thesis. I deeply admire the freedom of expression and thought that I was given while working under him, which helped me redefine my approach towards all aspects of life.*

I am much indebted to my co-guide ***Dr. Nihal Thomas****, Professor and Head, Department of Endocrinology, Christian Medical College, Vellore for his guidance, support and suggestions. His unflinching courage, prudence and conviction for accomplishing any task will always inspire me. I thank him for his considerate help and solace throughout.*

I take this opportunity to thank ***Dr. Simon Rajaratnam, Dr. H.S. Asha, Dr. Dukhabandhu Naik and Dr D.M. Mahesh,*** *Department of Endocrinology, Christian Medical College, Vellore****,*** *for their encouragement, guidance and kind support.*

A special thanks to ***Dr. Nitin Kapoor,*** *Department of Endocrinology, Christian Medical College, Vellore for his concern, help and motivation for executing this study.*

I would like to thank ***Dr. Joe Fleming, Dr. Victoria and Mr. Joseph****, Department of Clinical Biochemistry, Christian Medical College, Vellore for their help in rapid processing of the samples and for their kind co-operation throughout the study.*

A special thanks to all ***my Colleagues*** *of Department of Endocrinology, Christian Medical College, Vellore for their cooperation and enthusiastic help during the time of study tenure.*

I would also like to thank ***Dr. Antoni Samy****, for his help in processing the data for statistical analysis and giving it the precise configuration.*

*Very special thanks to **Ms Banu**, for her utmost help throughout the study from recruiting the subjects to formulating this thesis with utmost dedication and sincerity and for all the care and affection during my study period.*

*My appreciation also goes to **Mr. Kali, Mr.Thanigachalam, Mr.Magesh, Mr. Mohan, and Mrs.Sudha** for their cooperation and enthusiastic help during recruitment of subjects, sample collection and for always being amenable, whenever I needed their help.*

*My special thanks to all **staff members,** Department of Endocrinology, Christian Medical College, Vellore for their cooperation during the period of my research.*

*I take this opportunity to sincerely acknowledge **CMC Fluid Research** for providing financial assistance that helped me to perform my work comfortably.*

*No choice of words would suffice to adequately register my love and gratitude to my **Parents**. I thank them for their unconditional love, support and care. I take this moment to promise them that I would always keep up their faith in me. I also take this opportunity to express my admiration for my brother, who has always been my strength to fall back on.*

*It is impossible to express thanks in any words to my husband **Dr RaghavendraNayak**, who has been my confidence and kept me strong through all odds. I thank him for his encouragement to perform my best at each step.*

Most importantly, I owe my gratitude to all the subjects who consented to be a part of my study and made this work possible.

Last but not the least; I bow in gratitude to Almighty who has always blessed me with his choicest bounties. I thank Him for always guiding me through this endeavour.

All may not be mentioned, but no one is forgotten.

Sahana Shetty

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ALP	Alkaline Phosphatase
AUC	Area Under Curve
BMD	Bone Mineral Density
BMI	Bone Mass Index
BTM	Bone Turnover Marker
CV	Coefficient of Variation
CI	Confidence Interval
COL1 A1	Collagen 1 Alpha 1
CTX	C-Terminal Telopeptides Type I Collagen
CLIA	Chemiluminescent immunoassay
DXA	Dual Energy X-ray Absorptiometry
DPD	Deoxy Pyridinoline
DCI	Dietary Calcium Intake
DKKI	Dickkopf-related protein 1
ELISA	Enzyme linked immunosorbent assays
ECLIA	Electrochemiluminescentimmunoassay
FA	Forearm
FN	Femoral Neck
FRAX Tool	Fracture Risk Assessment Tool
ICMR	Indian Society for Bone & Mineral Research
IOF	International Osteoporosis Foundation
IFCCLM	International Federation of Clinical Chemistry & Laboratory Medicine

IPAQ	International Physical Activity Questionnaire
iPTH	Intact Parathyroid Homone
LS	Lumbar Spine
OR	Odds Ratio
OC	Osteocalcin
OPG	Osteoprotegerin
PA	Physical Activity
P1NP	Procollagen type I N-terminal propeptide
QCT	Quantitative Computed tomography
QU	Qualitative Ultrasound
RANKL	Receptor Activating Nuclear Factor Kappa B Ligand
ROC	Receiver Operating Curve
RIA	Radioimmunoassay
SD	Standard Deviation
SES	Socio Economic Status
SPPI	Secreted Phosphoprotein-1
TRACP5b	Tartrate resistant acid phosphatase isoform 5b
VDR	Vitamin D Receptor
WHO	World Health Organization
WnT	Wingless-NT
YSM	Years since Menopause
25(OH)D	25-Hydroxyvitamin D

Introduction

Osteoporosis is an important public health problem worldwide and is expected to increase with improved life span. The average life expectancy of an Indian woman is 68 years as per the WHO health statistics (2011) and by 2021, an increase to 73 years is expected.[1] Thus non communicable diseases like diabetes, hypertension, coronary artery disease and osteoporosis are on the rising trend with increasing longevity.

India has a high reported prevalence of osteoporosis; with one out of two women and one out of five men above the age of 50 years at risk of osteoporosis.[2,3] As per census 2011, the number of women in India is 586.5 million. About 100 million of them are in the postmenopausal age group. Osteoporosis has been reported in 50 percent of South Indian postmenopausal women.[2] When considering the population in the postmenopausal age group and half of them having osteoporosis, magnitude of problem seems to be huge with 50 million people estimated to have osteoporosis or low bone mass according to International osteoporosis foundation, Asia specific audit estimates.[4]

Osteoporosis is a silent disease till the occurrence of a fracture. Complications of osteoporosis in the form of fracture, the most dreaded being hip fracture contributes to increased morbidity and mortality in elderly population.[5] With the increase in the life span, osteoporotic hip fractures are expected to increase worldwide and around half of them being estimated to occur from Asia.[6]

The economic burden associated with the management of these fractures is considerably high.[3] Osteoporosis goes undiagnosed at an early stage due to factors like lack of awareness among the medical personnel and limited availability of DXA scanners. There are no uniform guidelines regarding screening and use of other tools like Fracture risk assessment **(**FRAX) in resource poor settings.

The current gold standard test for the diagnosis of osteoporosis is by bone mineral density (BMD) assessment using Dual energy X ray absorptiometry (DXA). Low BMD is a strong predictor of fracture at any site more specifically at the same site.[7]

Table-1: Predictive value of BMD in predicting the risk of fracture for every 1 SD decrease in bone mineral density below the age adjusted mean.[7]

BMD measurement site	Relative risk of fracture		
	Hip	Vertebrae	Forearm
Hip	2.6 (2.0 to 3.5)	1.8 (1.1 to 2.7)	1.4 (1.4 to 1.6)
Lumbar spine	1.6 (1.2 to 2.2)	2.3 (1.9 to 2.8)	1.5 (1.3 to 1.8)
Distal radius	1.8 (1.4 to 2.2)	1.7 (1.4 to 2.1)	1.7 (1.4 to 2.0)

However, about 50% of women who sustain a fragility fracture may not have BMD in the osteoporotic range.[8]

Hence there is a need for assessment of other risk factors of osteoporosis and also use of bone turnover markers (BTMs) which reflects the underlying bone turnover process.

Bone is a dynamic tissue which undergoes constant remodeling throughout the life span. Bone turnover markers provide an insight of the dynamics of bone

turnover. The peak bone mass is attained by third decade and is dependent on genetic and environmental factors like adequate nutrition, physical activity and systemic illness. Once peak bone mass is attained, there is a gradual decline in bone mass with advancing age, which is a dynamic process and is dependent on both bone formation and resorption.

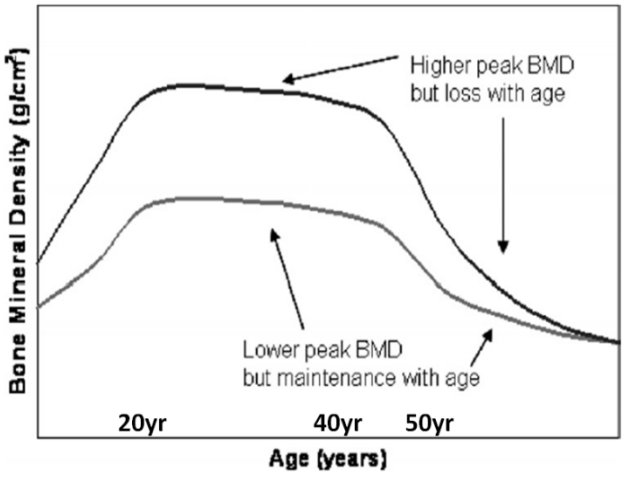


Figure-1: The influence of peak bone mass and rate of bone loss on BMD with age.[9]
(Modified from: Mitchell B D et al. J Clin Endocrinol Metab, May 2011)

Bone turnover is balanced with coupling of bone formation and resorption at various rates leading to continuous remodeling of bone. Age related and pathological state (e.g. corticosteroid use, hyperparathyroidism, inflammatory arthritis etc.) induced imbalance in the process of bone turnover may lead to accelerated bone loss. The resulting increased bone turnover leads to deterioration of bone microarchitecture and thus contributes to increased risk of fracture independent of low BMD. This microarchitectural alterations affecting the bone quality can be assessed by BTMs and thus serve as a complementary tool to BMD in the assessment of fracture risk.[10]

Bone turnover markers are important investigation tools for the diagnosis, treatment and monitoring of various metabolic bone disease, most importantly osteoporosis. Bone turnover markers (BTMs) may be utilized for assessing the response to treatment in short term after initiation of therapy for osteoporosis as BMD increment may not be evident if the DXA scan is repeated in 6-12 months. Various automated assays of bone turnover markers are available with manufacturer provided reference ranges. International Osteoporosis Foundation (IOF) and also International Federation of Clinical Chemistry & Laboratory Medicine (IFCCLM) have proposed serum CTX -1 and serum P1NP to be used as reference markers of bone resorption and formation respectively in clinical settings for assessment of fracture risk and monitoring therapeutic response to osteoporosis treatment.[11] Hence, we attempted to study the correlation of the BTMs with the BMD which is the current gold standard test in the management of osteoporosis.

There is a paucity of literature with regards to the normative data of bone turnover markers from India. The manufacturer provided reference range may not be the same for various ethnicities and may also depend upon various preanalytical variables like age, gender, race, diurnal and seasonal variations. In this study, we attempted to derive the reference range of the following bone turnover makers: serum C-Terminal Telopeptides Type I Collagen (CTX), serum Procollagen type I N-terminal propeptide (P1NP), serum Osteocalcin (OC) and urine Deoxy Pyridinoline (DPD) in healthy south Indian postmenopausal women and their daughters.

Osteoporosis is a multifactorial disease with various modifiable and non-modifiable risk factors contributing to the pathogenesis of osteoporosis. Genetic influence plays an important role in the acquisition of peak bone mass, the rate of

bone loss and the risk of osteoporotic fracture in an individual. Parental hip fracture is shown to be one of the major risk factor for fragility fracture. The familial predisposition to the fracture risk could be secondary to both genetic susceptibility and the environmental risk factors shared by the family members. Genetic influence on osteoporosis is complex with multiple candidate genes implicated to have small to moderate effects in the attainment of peak bone mass and bone loss.[12] Hence, we attempted to study the difference in bone mass and BTMs in daughters whose mothers had osteoporosis.

Apart from genetic and other non-modifiable factors, bone mass is also determined by various modifiable life style factors. Life style factors comprise of nutritional factors like calcium, vitamin D, proteins and the behavioural factors which includes physical activity, smoking and chronic alcoholism. The literature on the risk factors for osteoporosis in Indian subjects is limited. Hence, we studied various factors which would influence bone health in daughters and mothers who were representative of premenopausal and postmenopausal women from the community.

Low BMD is a proxy indicator for osteoporotic fracture which can be objectively measured. Hence, we looked at the influence of dietary calcium intake, the physical activity, parity, socioeconomic status on the bone mineral density in pre and postmenopausal women. We also looked at the association of these factors with BTMs.

Aims & Objectives

Aim of the study

To study the Bone health (including Bone Mineral Density and Bone Turnover Markers) and factors influencing them in postmenopausal women and their premenopausal daughters of urban Vellore in Southern India.

Objectives

- To study the correlation between BTMs and BMD (Bone mineral density) at various sites.
- To study the reference range for various bone turnover markers (BTMs) in healthy premenopausal and postmenopausal women.
- To compare the BMD and BTMs among daughters of mothers with osteoporosis with those whose mother's did not have osteoporosis.
- To look at various factors which influence BMD and BTMs in premenopausal and postmenopausal women.

Review of Literature

- **Introduction to Osteoporosis**
- **Magnitude of osteoporosis in Indian population**
- **Incidence and economic burden of osteoporotic fractures**
- **Peak bone mass**
- **Osteoporosis –definition**
- **Modifiable and Non modifiable risk factors pertaining to bone health**
- **Calcium, Vitamin D status and BMI**
- **Physical activity**
- **Socioeconomic status**
- **Tools for diagnosing osteoporosis**
- **DXA Scan**
- **Bone turnover markers**
- **Assays for Bone turnover markers**
- **Factors determining pre-analytical variability of BTMs**
- **Role of Bone markers in the assessment of fracture risk**
- **BTMs and osteoporotic treatment monitoring**
- **Ethnicity specific reference range for BTMs**
- **Primary prevention of osteoporosis**
- **Secondary prevention of osteoporosis**

Introduction to osteoporosis

Osteoporosis is the most common metabolic bone disorder characterized by a structural deterioration of bone tissue leading to an increased risk of fracture.[2] Osteoporosis is an important public health problem worldwide and is expected to increase with an improved life span.

Osteoporosis being a silent disease may present with dreaded complication like hip fracture if periodic screening and preventive strategies are not taken in the postmenopausal women and elderly men. Hip fracture secondary to osteoporosis contributes to an increased morbidity and mortality in elderly population. Osteoporosis (a state of low Bone Mineral Density) has been reported in about 50% of healthy Indian postmenopausal women.[1,2]

Bone mineral density (BMD) assessment using Dual-energy X-ray absorptiometry (DXA) scan is the current gold standard test for the diagnosis of osteoporosis.[13] However, DXA scan has its own limitations being a static measure and an expensive investigation with limited availability in many parts of our country.

BTMs are novel tools which provide insights into the dynamics of the different phases of bone remodeling. The wider availability of reliable, cost effective, sensitive and specific assays for bone turnover markers (BTMs) would complement the measurement of BMD in the management of osteoporosis especially in the follow up of these subjects who had been on antiresorptive or bone formation therapies.[10]

Magnitude of osteoporosis in Indian population

The prevalence of osteoporosis in an ambulant south Indian postmenopausal population shown by a study published in 2008 was 48% at the lumbar spine, 16.7% at the femoral neck, and 50% at any site. In addition, over half of study population also had vitamin D deficiency.[1] Around 50 million postmenopausal women in India are estimated to be affected by osteoporosis.[14]

Incidence and economic burden of osteoporotic fractures in India

Untreated osteoporosis in postmenopausal women and elderly men can result in a fracture usually following a trivial fall. Common sites of osteoporotic fractures include wrist, spine and hip which carry significant morbidity and mortality.

In a north Indian study, the crude incidence of hip fracture was 129 per 1 lakh population with further subdivision showing 105 per 1 lakh in men and 159 per 1 lakh in women, which is similar to studies from other Asian countries.[15] Incidence of hip fractures are shown to vary with the ethnicities and geography.[16]

Osteoporotic fractures in Indians seem to occur 10-20 years earlier as compared to Caucasians.[15] In a study by Dhanwal DK et al, the mean age of hip fracture in Indian women was 63.6±9.9 years.[17] Vitamin D deficiency and secondary hyperparathyroidism have been reported in about two thirds of the hip fracture subjects.[15]

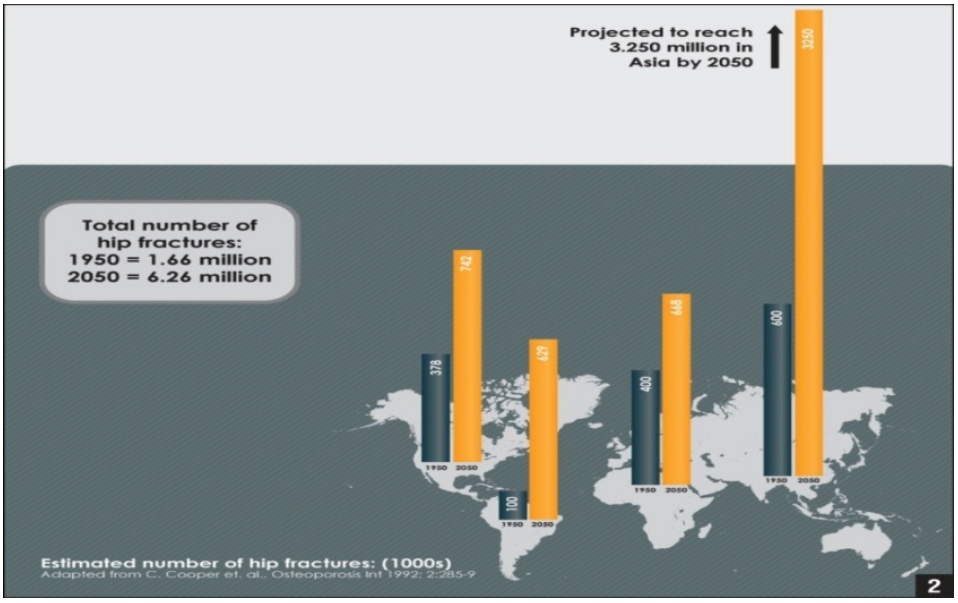


Figure-2: Worldwide prevalence of osteoporotic hip fractures[18] *(adapted from Shuler FD et al. Orthopedics. 2012).*

It is estimated that worldwide over 200 million people have osteoporosis. The health care services costs are already considerable and are predicted to double by 2050 based on the current trends.[17] The life expectancy of an Indian woman is 68 years as per WHO health statistics (2011) and by 2021, an increase to 73 years is expected.[1]

Osteoporotic fractures are worrisome to the patient and the family due to the associated increased mortality, morbidity and poor quality of life. They also carry a huge economic burden to the society and nation as a whole. The minimum expenditure incurred in the treatment of an osteoporotic hip fracture is around 90,000 rupees, which will have a significant impact on the economy, considering the number of osteoporotic subjects in the Indian community.[3] Therefore, osteoporosis is a significant problem in the elderly and its prevalence in the society is expected to increase as the ageing population in the community is on rise.

In a follow up study of postmenopausal subjects (n=104) with surgically treated hip fracture from southern India, one year mortality was 4 times (20% versus 5%) more than age and BMI matched controls.[5] The mortality of a patient after sustaining a hip fracture increases by 24 to 30% within one year of the hip fracture.[19,20]

Of those who survive, almost 50% are permanently incapacitated and about 20% need long term nursing home care.[21] This not only adds on to the morbidity but also to the cost of health care at the patient, community and national level. The mortality, morbidity and cost associated with osteoporotic fractures are reduced to a great extent by early identification of risk factors and treatment of osteoporosis in the high risk group in addition to maintaining recommended calcium and vitamin D nutrition.[22]

Peak bone mass

"Peak bone mass is defined as the amount of bone acquired at the end of maturation of skeleton".[23]

The acquisition of peak bone mass occurs by about 30 years of age after which there is a gradual decline in the bone mass. Bone turnover occurs at a maximal rate at birth with both high bone formation and resorption, after which, it rapidly declines. The bone mass is gradually increased through child and adolescent phases with peak bone mass generally achieved by third decade of life, after which, there is constant resorption of bone at slow rate, which may be exacerbated with estrogen deficiency as in menopause or in other secondary causes of osteoporosis.

Both genetic and environmental factors determine the peak bone mass. Nutritional factors like calcium, vitamin D, protein and physical activity are important environmental factors which determine the bone mass achieved.[24]

The two important determinants of bone strength are bone density and bone quality.

a) Bone mineral density

Bone density is expressed as density of bone mineral per area or volume. Bone mineral density of an individual depends of peak bone mass acquired during first three decades of life which is influenced by the genetic and environmental factors and rate of bone loss. Various techniques are available for the measurement of BMD, which is used to define osteoporosis. Indian subjects seem to have low BMD as compared to Caucasians in view of smaller skeletal frame, vitamin D deficiency, early menopause, poor sunlight exposure.[25]

b) Bone quality:

Bone quality depends on micro architecture of bone, trabecular connectivity, size of cortical and trabecular bone which determine morphometry and dynamic processes occurring in the skeleton like bone turnover, micro-fracture accumulation and mineralisation.

Osteoporosis

Osteoporosis is defined as "a condition characterized by a low bone mass and microarchitectural deterioration of bone tissue leading on to increase in the bone fragility and predisposing to fracture".[26]

Osteoporosis is defined based on the bone mineral density (area density in g/cm^2) by using T scores and Z scores.

T score: Number of standard deviations above or below BMD of age matched control
Z score: Number of standard deviations above or below BMD of young normal mean

WHO definition of osteoporosis: (based on Bone mineral density) is as follows:

Osteoporosis	**T score : ≤ -2.5**
Osteopenia	**T score : 1- to -2.4**
Normal	**T score : > -1**

For premenopausal women, a term "low bone mass" is used to describe a subject who's BMD Z score is less than minus 2.[27]

In a meta-analysis by Marshall D et al, the relative risk of hip fracture for 1 SD decline in the bone mineral density at hip was 2.6.[7] The predictive ability of decline in BMD for sustaining a fracture is comparable to increased BP for stroke and Hypercholesterolemia for cardiovascular disease.[28]

On the other hand, among those who sustain a fracture, 50% women and 70% men do not have BMD in the osteoporotic range.[8] There are several factors which influence bone mineral density either favorably or adversely (Table-2).

Table -2: Modifiable and Non modifiable factors influencing bone health

1. Genetic factors
2. Nutritional factors **a)Calcium b) Vitamin D c)Protein d) role of BMI**
3. Physical activity
4. Socioeconomic status
5. Hormonal factors – sex hormones deficiencies, thyroid hormone and glucocorticosteroid excess, parathyroid hormone excess , growth hormone excess or deficiency etc
6. Chronic systemic diseases like chronic liver disease, chronic kidney disease, malabsorption syndrome
7. Inflammatory conditions like inflammatory arthritis, inflammatory bowel diseases, connective tissue disorders etc
8. Smoking
9. Alcoholism
10. Medications: glucocorticoids, calcineurin inhibitors, antiretroviral drugs, selective inhibitors of serotonin reuptake, anticonvulsants, loop diuretics, heparin, oral anticoagulants, and proton pump inhibitors

Genetic factors

Genetic factors play an important role in determining peak bone mass as well as rate of bone loss of an individual. Genetic factors like estrogen receptor alpha and vitamin D polymorphism, COL1 A1, SPP1 have been shown to be associated with low bone mass.[29] The other candidate genes implicated in low BMD and rapid bone loss based on genome wide studies and linkage analysis is related to one of the below pathways:

1. Receptor activator of nuclear factor k B –RANK lignad- osteoprotegerin (RANK/RANKL/OPG pathway)
2. Wnt B catenin pathway
3. Estrogen endocrine pathways
4. Chromosome 1p36 [9]

Epigenetic modification in RANK/RANKL/OPG pathways and WNT/ β catenin pathways through DNA methlyation, micro RNA expression and post translational modification of histones are also postulated to contribute to pathogenesis of osteoporosis.[30]

Parental hip fracture is one important factor which increases the fracture risk in the offspring and thus used in in FRAX, highlighting the influence of the genetic factors on BMD.

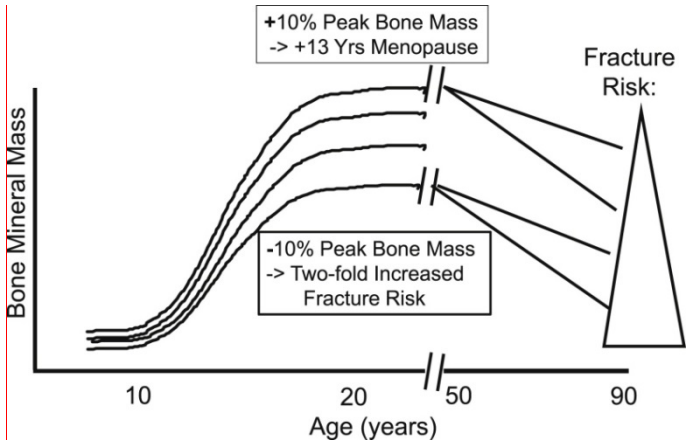


Figure- 3: Role of peak bone mass on fracture risk[24] *(adapted from Rizzoli et al. Best Practice & Research Clinical Endocrinology & Metabolism. 2014)*

Calcium, Vitamin D status and BMI

Dietary calcium, vitamin D and proteins are well established nutritional factors positively influencing bone health. They help not only in accruing bone mass and maintaining bone mass but also reduces the rate of bone loss.

a. Dietary calcium and protein

Higher calcium and protein intake result in accrual of higher peak bone mass. About 5% increase in peak bone mass may result in 50% reduction in fractures, thus highlighting the importance of peak bone mass.[24] Lee at al showed a positive correlation between daily calcium intake, phosphorus intake, calcium / phosphorus ratio and BMD both at the spine and femoral neck in postmenopausal women and only at the femoral neck in premenopausal women.[31] Many epidemiological studies have documented low dietary calcium intake in India. Daily dietary calcium intake of less than 400 mg per day has been reported in previously published studies from

South India.[1] Indian council of medical research recommends a daily calcium intake of 1000mg/day in premenopausal women and 1300mg/day in postmenopausal women.[32]

Protein intake and physical activity have been shown to increase the bone strength by increasing the cross sectional area and also by increasing the number of trabeculae.[33]

IGF1 is another important factor which not only determines the longitudinal growth but also in bone matrix mineralisation by stimulating the transport of inorganic phosphate into osteoblastic cell linages, renal tubular resorption of phosphate and calcitriol synthesis. The synthesis of IGF1 in osteoblastic lineages is stimulated by dietary proteins like arginine, thus implicating the role of proteins in bone health.[34,35]

b. Vitamin D deficiency

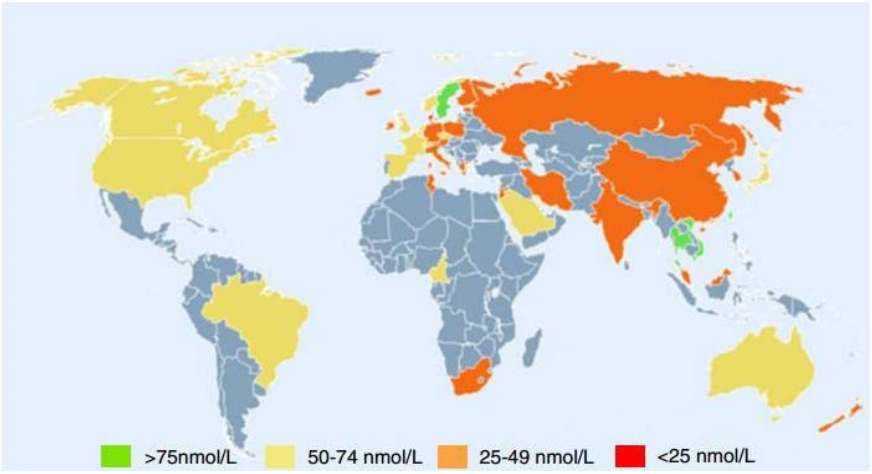


Figure-4: Prevalence of vitamin D deficiency across the globe[36] *(adapted from:Wahl DA et al Arch Osteoporos. 2012)*

A higher prevalence of vitamin D deficiency has been shown in all age groups in India.[37] About 50 % prevalence of vitamin D deficiency and insufficiency in cohort of healthy postmenopausal women has been reported from south India.[1] Subjects with vitamin D deficiency had a significantly lower BMD at femoral neck (0.657 vs 0.694, p=0.03).[1]

Vitamin D deficiency contributes to low bone mineral density by:

a. Decreasing the bone mineral content by reducing the mineralisation and
b. Increased bone resorption as a result of secondary hyperparathyroidism.[37]

c. Body Mass Index (BMI)

A high BMI has been considered as a protective factor in terms of osteoporotic fractures, mainly for the hip and pelvis fracture. However the relationship between BMI and BMD has raised controversies in recent times as few studies showed increased risk of leg and ankle fracture in obese subjects.[38]

A recent meta-analysis of relationship of fracture and BMI, showed a complex association with increased risk of hip fracture with low BMI when adjusted for BMD, however it was protective for osteoporotic fractures involving tibia, fibula, distal forearm and upperarm.[39]

Physical activity (PA)

PA has been associated with higher BMD and reduction in fracture incidence through different roles across the age groups. PA in children and adolescents promote incurring maximum peak bone mass, in adults, reduces the age related bone loss and reduces the risk of falls by increasing muscle strength and improving the

neuromuscular function in elderly.[40] Physical activity has a protective effect in reduction of fracture risk both by increase in BMD as well as by decreasing the risk of fall by promoting muscle strength and coordination.[32] Subjects with higher level of physical activity has been shown to have a higher BMD at hip.[41]

Physical activity can be assessed using tools like self-reported questionnaires, direct observation, indirect calorimetry, heart rate telemetry and movement sensors like accelerometers. International Physical Activity Questionnaire (IPAQ) is an internationally validated self-reported questionnaire for assessment of physical activity.[42] IPAQ short and long forms have been validated across various population and age groups,[43] however is limited by lengthy questionnaire and over estimation in some studies.[44]

Socioeconomic status (SES)

Postmenopausal women from lower socioeconomic status are shown to be at increased risk of osteoporosis and fragility fractures which may be secondary to poor nutrition with respect to protein and dairy products.[45]

SES has been shown to have significant association with the ten year risk prediction of hip fracture in postmenopausal women as assessed by FRAX.[46]

Among the healthy school children from North India, those in lower socioeconomic strata were found to have a lower BMD and vitamin D levels as compared to higher socioeconomic strata children. There was also a significant association between BMD and age, height, weight and nutrition.[47]

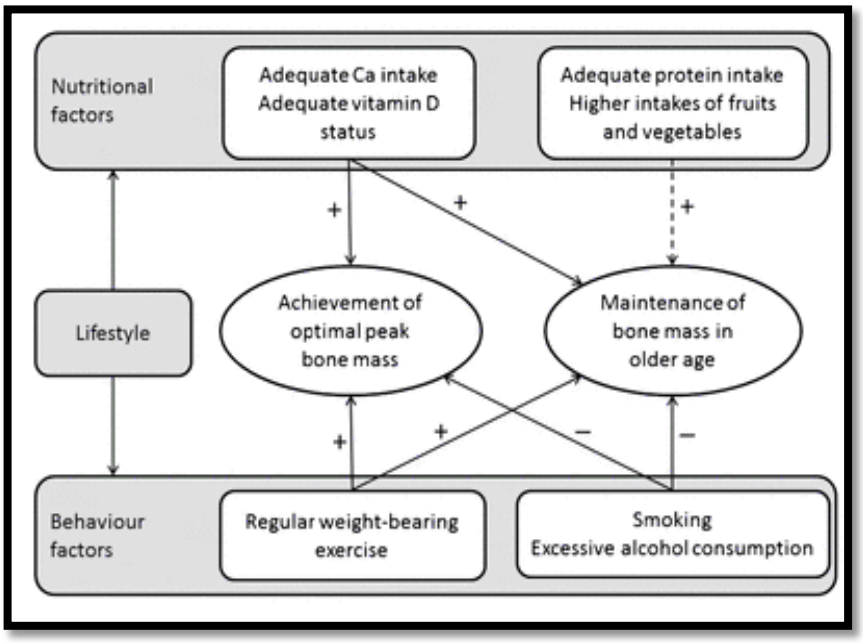


Figure-5: Life style factors affecting bone health and other risk factors [48]*(adapted from Zhu K et . Curr Osteoporos Rep. 2015)*

Secondary causes of osteoporosis include systemic disorders which lead to either increased bone resorption or decreased bone formation. The endocrinopathies like hypogonadism, thyrotoxicosis, hyperparathyroidism, growth hormone excess or deficiency etc, chronic liver disease, chronic kidney disease, chronic inflammatory diseases like inflammatory arthritis, inflammatory bowel diseases, connective tissue disorder, chronic alcoholism, smoking and medications like corticosteroids affect the bone remodeling.

Fracture risk assessment tool (FRAX): Web based tool which incorporates various risk factors of osteoporosis in addition to BMD in predicting the ten year risk of major osteoporotic fracture and hip fracture.[49]

Tools for diagnosing osteoporosis

Diagnosis of osteoporosis is based on assessment of bone mineral density using various assessment tools like:

- DXA scan
- Single energy X ray absorptiometry (SEXA) scan
- Quantitative Computed tomography (QCT)
- Qualitative Ultrasound
- Single photon absorptiometry
- Double photon absorptiometry

DXA Scan

Among the various modalities currently available to diagnose osteoporosis, assessment of the BMD by DXA is gold standard in clinical practice for the past two decades.

Diagnosis of osteoporosis by DXA scan is by comparing the subjects BMD with the normative data provided in the scanners. Most DXA scanners have incorporated Caucasian based normative data. The ethnicity specific normative data are also available for various populations. The performance of Indian council of medical research reference data (ICMR data) was compared to Caucasian reference data (Hologic Data) in Indian subjects and was found that even though there was an almost perfect agreement in diagnoses of osteoporosis between the two databases, 23.5% hip fracture subjects defined to have osteoporosis by HD were classified by ICMRD to have osteopenia.[50]

Advantages of DXA scan over other modalities:

1. Diagnosis of osteoporosis by using T-scores derived by BMD comparison with available normative data
2. Reliable prediction of fracture risk as proven by various studies
3. Good precision and stable calibration
4. Good instrument quality control measures
5. Shorter scan times
6. Effective for follow up of osteoporosis treatment
7. Minimal radiation exposure
8. Noninvasive

Limitations:

1. Bone quality cannot be assessed which is also a important determinant of bone strength.
2. Artifacts causing errors in BMD measurements by DXA like degenerative diseases, extraneous calcification, metal implants, metastasis, barium contrast media, radionuclide examinations
3. 2D assessment is affected by bone size and shape
4. Interference by soft tissue composition
5. High cost and availability
6. Inability to detect a change over shorter duration of follow up (<12-18months in common clinical conditions)

In view of this, bone turnover markers are gaining widespread acceptance in the management of metabolic bone disease, most importantly osteoporosis.

Bone turnover markers

Bone remodeling –Physiology of Bone turnover markers:

Bone is a dynamic tissue structure which undergoes through phases of remodeling throughout once life time. After achieving peak bone mass, bone undergoes constant remodeling through bone resorption followed by bone formation sequentially at "Bone remodeling unit", which is a basic multicellular unit of bone.

The initial process of bone resorption is osteoclast mediated formation of resorption pits by the dissolution of bone mineral and matrix which result in the release of bone matrix components into the blood stream. This is followed by phase of bone formation phase by osteoblasts which fill in the resorption cavity and cause mineralisation. The biochemical substances released into systemic circulation during these phases of bone resorption and formation are called bone turnover markers.[51] Under normal circumstances, bone resorption takes place in about ten days and subsequent bone formation in about 3 months.[52] Upto 20 percent of bone may be replaced by remodeling every year. These two activities complement each other and are brought about by bone cells namely the osteoclasts, osteoblasts and osteocytes under the regulation of systemic (Parathormone, PTH related peptide and vitamin D) and local mediators(cytokines like interleukins, prostaglandin E2 and growth factors).[53]

The bone formation and resorption are tightly coupled processes with osteocyte being the most important regulatory cell which responds to various mechanical stresses by releasing signals to osteoblasts and osteoclasts to initiate the process of bone formation and resorption.[54]

The bone remodeling is mainly mediated by RANKL/RANK /OPG mediated pathways.[55]

The bone markers currently available include enzymes and non-enzymatic peptides derived from the cellular and non-cellular compartments of bone.

The bone turnover markers are grouped into 2 categories based on the metabolic phase during which they are produced as:

1) Bone resorption markers
2) Bone formation markers

Table-3: Bone turnover markers:

Bone formation markers **1) Osteocalcin (OCN)** **2) Bone specific alkaline phosphatase(bone ALP)** **3) N - terminal propeptide of type 1 procollagen (P1NP)** **4) C – terminal propeptide of type 1 procollagen (P1CP)**
Bone resorption markers **1) C- terminal telopeptide type 1 collagen (CTX -1/CTX)** **2) N- terminal telopeptide type 1 collagen (NTX -1/NTX)** **3) C- terminal telopeptide type 1 collagen** **4) generated by matrix metalloproteinase(CTX -MMP/ICTP)** **5) Helical peptide 620-633 of the α 1 chain** **6) Deoxypyridinoline (DPD)** **7) Tartrate resistant acid phosphatase isoform 5b (TRACP5b)**

A limitation of this classification is that some BTMs like hydroxyl proline and osteocalcin may represent both formation and resorption phases. Also they may have extra skeletal origin and are not always from the bone. Finally these markers are not disease specific and only represent bone remodeling. [56]

IOF and IFCCLM have proposed serum CTX -1 and serum P1NP to be used as reference markers for the assessment of fracture risk and monitoring therapy in clinical settings.[11]

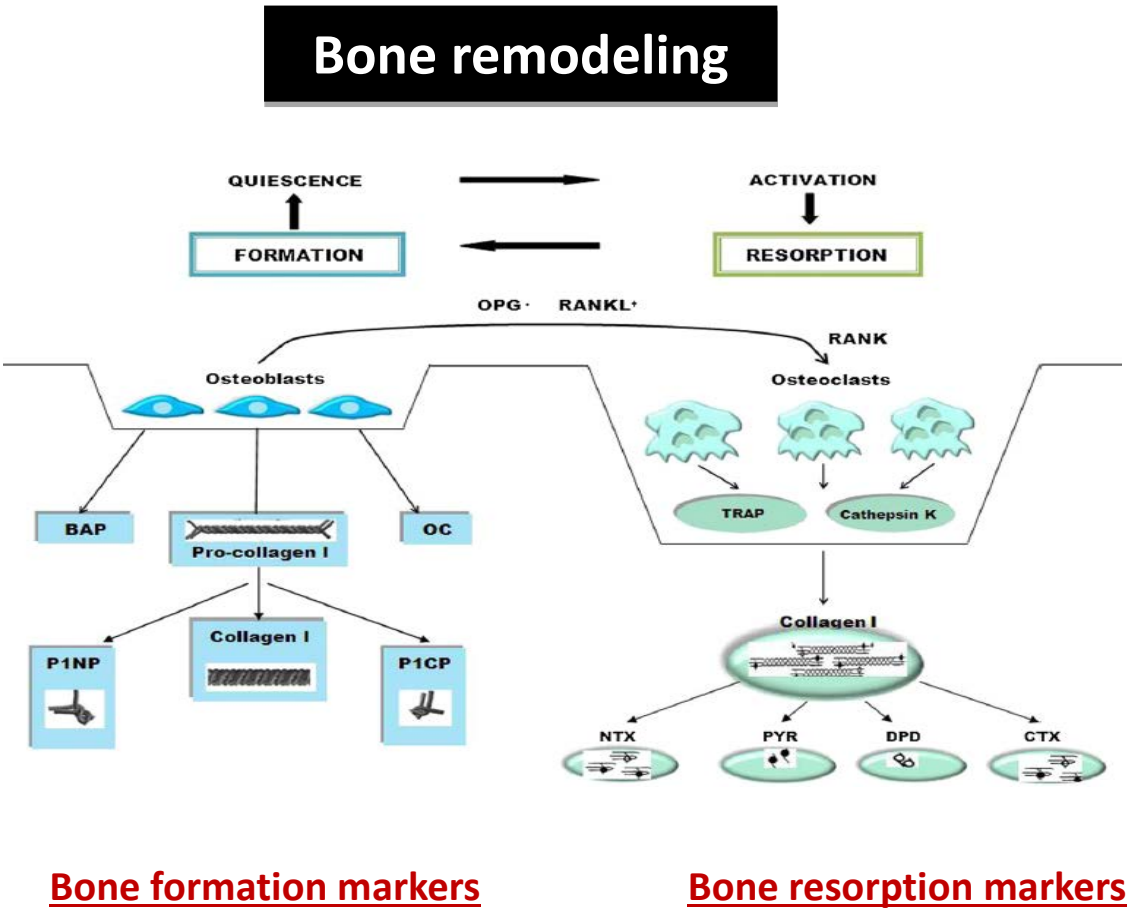


Figure- 6: BTMs in different phases of bone remodeling[10]
(*adapted from: Wheater G et al. Journal of Translational Medicine 2013)*

A) Markers of bone resorption

These are formed during the phase of bone resorption involving osteoclastic activity. This includes byproducts of osteoclasts released during bone resorption and osteoclastic enzymes.

The bone resorption markers are categorised as follows:

1) **Collagen degradation products: Hydroxyproline**

   Pyridinium crosslinks (Pyridinoline, deoxypyridinoline)

   Telopeptides of type 1 collagen (Cterminal:CTX -1, CTX –MMP, N terminal: NTX-1)

2) **Non collagenous proteins**

   Bone sialoprotein

3) **Osteoclastic enzymes**

   Tartarate resistant acid phosphatase

   Cathepsin K

1) Carboxy terminal crosslinked telopeptides of Type 1 collagen (CTX - Beta cross lap)

CTX are degradation products of Type 1 collagen of bone. These non-helical fragments containing cross linking regions are the carboxy terminal telopeptides of the type 1 collagen, generated by the activity of the enzyme cathepsin K. The native CTX exists in two forms: α and β isomerised forms. These isomerised forms undergo further isomerisation to form D and L forms. The spontaneous β isomerisation of α isoforms occurs with protein aging. Hence the altered ratio of : α and β isomerised forms occurs with new bone formation as in physiological conditions like growing

children and pathological conditions like malignant bone diseases, paget's disease of bone and those receiving Parathyroid hormone treatment.[57,58]

The major problem with CTX measurement is its circadian variation, with peak in the second half of night and nadir in the afternoon. Studies looking at the circadian variation of CTX reported a peak level of CTX at 05.00 hr and nadir at 14.00 hr.[59,60] CTX measurements is also affected by food intake with reduction in serum levels by 20% postprandial as compared to fasting state. Hence to reduce this preanalytical variability, it is recommended to collect the sample in the morning after an overnight fast. [61]

2) Amino terminal crosslinked telopeptides of Type 1 collagen (NTX)

They are generated from the amino terminus of the type 1 collagen by cleavage of N terminal region by cathepsin K during resorption phase of bone turnover. NTX is measured using a monoclonal antibody against specific N terminal epitope. NTX levels are usually altered in liver and renal failure. Urine NTX exhibit less circadian and postprandial variability as compared to CTX, However, 24 hour urine collection is more cumbersome.[62]

3) Pyridinoline (PYD) and Deoxypyridinoline (DPD)

These are covalent pyridinium compounds formed during the fibrillar collagens maturation. These crosslinked collagens released into the circulation when mature type 1 collagen is proteolytically degraded for mechanical stabilisation of the molecule. They reflect the degradation of mature cross linked collagens and is measure newly synthesized collagens. In addition, the urinary excretion of DPD is not influenced by dietary sources as they are not absorbed from the gut and are

highly specific for skeletal tissues. While PYD is found in cartilage, bone, ligaments and vessels, DPD is almost exclusively found in bone and dentin. These can be measured in a 24 hour urine collection or as creatinine corrected spot urine measurements Thus, the PYD and DPD are one of the good indices for bone resorption.[63]

Novel biomarkers: Tartrate-Resistant Acid Phosphatase (TRAP, TRAcP5b), Cathepsin K, Receptor Activator of Nuclear factor Kappa B Ligand (RANKL), Osteoprotegerin(OPG) , Dickkopf-related protein 1 (DKK1) and sclerostin

These are novel biomarkers of bone resorption currently used in research setting to study bone remodeling and the efficacy, safety, mechanism and mode of action of drugs used in osteoporosis and other metabolic bone diseases.

TRACP5b is the isoform of acid phosphatase which is resistant to degradation by tartarate, cleaved by protease into isoform 5b, most specifically expressed in the ruffled border of osteoclast and cleaves type 1 COL into fragments during bone resorption.[64]

Cathepsin K is an osteoclastic enzyme, cysteine proteinases present at active osteoclasts ruffled border, which is specific of osteoclastic activity.[65]

RANKL are osteocytes markers which reflect bone microenvironment. They are produced on activation of osteoclasts by activated by B and T cells and stimulate the differentiation and activity of osteoclasts after binding to RANK.

Osteoprotegerin is another marker of osteocyte activity, synthesized by osteoblasts and act as decoy receptor to RANKL. They prevent osteoclastogenesis by

binding to RANK and thus reduce bone resorption. Dickkopf-related protein 1 (DKK1) and sclerostin are markers of osteocyte activity secreted by osteocytes and inhibit bone formation by inhibiting WNT signalling by binding to LRP -5 in the osteoblasts.

B) Markers of Bone Formation

Bone formation markers are products of active osteoblasts expressed during different phases of their development and bone formation. They are considered to reflect different aspects of osteoblast function and bone formation. All markers of bone formation are measured in serum or plasma.

Bone formation markers are categorised as:

- **By products of collagen synthesis**
  Propeptides of type 1 collagen: (C terminal: P1CP, N terminal: P1NP)
- **Matrix proteins**
  Osteocalcin
- **Osteoblast enzymes**
  Alkaline phosphatase (total and bone specific)

1) Procollagen Type I Propeptides

PINP and PICP are peptides produced from type I procollagen by proteases mediated posttranslational cleavage at N and C terminal respectively. PINP and PICP are predominantly synthesized from bone (proliferating osteoblasts and fibroblasts) followed by tendon, dentin skin, and cartilage.[66] Since skeletal tissues undergo a higher rate of turnover than non-skeletal tissues, they contribute a preponderance of collagen propeptides to circulation.[66] PINP is cleared by the mannose receptor, which

in turn can be regulated by growth hormone and thyroid hormones, thus complicating interpretation in subjects with pituitary or thyroid dysfunction. P1NP exists in serum as trimeric form or its thermal degradation product product in the monomeric form. Immunoassays detect either the trimeric forms (automated IDS ISYSS assays) or both forms which is called total P1NP assays (automated Roche Elecsys assay).[67]

Various studies using P1NP have shown a low intra-individual variability, lesser circadian variability, stability at room temperature and good assay precision. P1NP is proposed as a reference bone formation marker by IOF in view of reliability of P1NP assays and its response to treatment .[11]

2) Serum Alkaline Phosphatase (total and bone specific):

Alkaline phosphatase is a ubiquitous, membrane-bound tetrameric enzyme present in the plasma membrane of the osteoblasts. Its major function is formation of osteoid and mineralisation mediated by enzymatic degradation of pyrophosphate, an inhibitor of mineralisation, at an alkaline pH.[68] ALP is the first bone turnover marker utilised in both research and clinical practice.

3) Osteocalcin (OC):

OC is a hydroxyapatite-binding protein exclusively synthesised by osteoblast, odontoblasts and hypertrophic chondrocytes. It is also called as the bone gla protein and constitutes 15% of the non-collagenous bone matrix. OC is an active molecule involved in the organization of the extracellular matrix which is bound to hydroxyaptite in the mineralised matrix of bone and is released during both bone resorption and formation.[69]

OC is considered a specific marker of osteoblast function. Mineral binding of osteocalcin requires γ carboxylation of three glutamate residues of OC. The undercarboxylated OC has been shown to have a negative correlation with hip fracture in elderly women. The undercarboxylated OC has also shown to have effect on metabolic processes like increasing insulin secretion and action.[70]

Being a late marker of osteoblastic activity, it has been labelled as a bone formation marker but is limited by short half-life, unstable intact molecule and influence of vitamin K, renal function and variations associated circadian rhythm. OC has been found to be a useful biomarker in steroid induced osteoporosis.[71]

Bone turnover markers assays

Various methods available for measurements of bone turnover markers include:

1. **Radioimmunoassay (RIA)**
2. **Immunoradiometric assays (IRMA)**
3. **Enzyme linked immunosorbent assays (ELISA)**
4. **Chemiluminescent immunoassay (CLIA)**
5. **Electrochemiluminescent immunoassay (ECLIA)**

Factors determining preanalytical variability of BTMs:

BTMs exhibit significant intraindividual and preanalytical variability. These variability must always be kept in mind while interpreting the BTMs in clinical scenario. Thus various factors affecting BTMs need to looked for, while interpreting BTMs.

Table-4: Factors affecting BTMs[72]

I. **Non modifiable factors**

Age

Sex

Menopausal state

II. **Modifiable factors**

Circadian variation

Seasonal variation

Fasting and food intake

Physical activity

Menstrual variation

Vitamin D deficiency

Secondary hyperparathyroidism

Chronic renal disease

High bone turnover diseases: Thyrotoxicosis, primary hyperparathyroidism, Acromegaly, bone metastasis, paget's disease**.**

Low bone turnover diseases: Hypothyroidism, hypoparathyroidism, hypopituitarism, GH deficiency

Dissociation between bone formation and resorption: Cushing's disease and multiple myeloma

Recent fracture

Chronic diseases with limited mobility: Stroke, paraplegia, hemiplegia, dementia, schizophrenia, depression

Drugs: Corticosteroids, oral contraceptives, aromatase inhibitors, antiepileptics, heparin, thiazolidinediones, vitamin K antagonist

In view of analytical and pre analytical variability of BTMs assays, appropriate sample collection and storage conditions must be followed. To reduce the biological variability, samples must be collected in fasting state. Subsequent serial measurements must be done on the same time and same season to account for the diurnal and seasonal variation.

The preanalytical variability can be reduced by appropriate sample collection with proper preparation of patient and by standarised specimen handling, storage and specimens. For BTMs with predominant renal excretion (ex:OC), correction for renal function is required.

Changes in the BTMs must be large for monitoring clinical response in view of biological and analytical variations. While interpreting the BTM response "Least significant change" (LSC) for each BTM must be utilised which is derived by product of the each BTMs precision error provided by the laboratory by 2.77(95% confidence interval).[73]

A. Role of BTMs in assessment of fracture risk

The high BTMs may predict the risk of sustaining osteoporotic fracture in postmenopausal women independent of BMD. State of increased bone turnover leads on to deterioration of bone micro architecture and thus contributes to low BMD and also an increased risk of fracture. This micro architectural alteration affecting bone quality can be assessed by BTMs and thus serve as a complementary tool to BMD measurement in assessing the fracture risk. The state of increased bone turnover markers also affect the structural integrity of bone as the newly synthesised bone

may be less mineralised with decreased post translational modification in terms of decreased beta cross links and beta isomerisation of the type 1 collagen.[74,75]

i. **OFLEY study (Os des Femmes de Lyon)**

Garnero P et al, studied 435 healthy untreated younger postmenopausal women aged 50–89 years (mean, 64 years) from OFLEY cohort comprising 1039 women (31–89 years of age). Baseline bone markers were compared in 55 women who had sustained fractures(20 vertebral and 35 peripheral fractures) and 380 controls who were followed up for 5 years. Two fold increased risk of fracture was seen in women with BTMs in highest quartile with relative risk of 1.8(CI: 1-3.4) for urinary free pyridinoline, 1.7 (CI:0.9-3.2) for urinary NTX, 2.3 (CI:1.3–4.1) for urinary CTX, 2.1 (CI:1.2–3.8) for serum CTX, 2.4 (CI:1.3–4.2).[76]

ii. **The Rotterdam study**

Urinary pyridinium cross links (including total pyridinoloine, free pyridinoline, total deoxypyridinoline and free deoxypyridinoline) have been shown to have a significant association with hip fracture risk with age adjusted RR of 3.3, 3, 2.2, 1.8 respectively in a nested case control.[77]

iii. **EPIDOS (Epidemiologie de l'Ostéoporose) prospective cohort study**

Vergnaud P et al studied 104 subjects over 75 years of age with hip fracture versus 255 controls from a cohort of 7598 postmenopausal women. They found that under carboxylated osteocalcin measured by ELISA predicted the increased fracture risk with a odds ratio of 1.9 (1.2-3.0), which persisted even after adjusting for femoral neck BMD and mobility [adjusted OR- 1.8(1.0- 3.0)].[78]

iv. **Nested case control study from EPIDOS cohort on serum CTX**

In a nested case control study from EPIDOS cohort of postmenopausal women, which included 115 fracture subjects as cases and 293 controls, serum CTX samples collected in the afternoon had a significant prediction of fracture, with hazard ratio of 1.8(1.01- 3.76), unlike whole group CTX which was not predictive.[79]

The most useful application of BTMs is in monitoring the compliance and adherence to treatment. Adherence to treatment is the most challenging aspect in the treatment of osteoporosis, especially for medication which requires strict precautions, dosing, schedule and parental route of administration. Thus, BTMs could identify poor compliance and therapeutic efficacy. Therefore, BTMs need to be measured before starting treatment and subsequently at follow ups.[80]

Improving Measurements of Persistence on ACtonel Treatment (IMPACT) study

In a multinational prospective, open-label, cluster-randomized study of postmenopausal women(IMPACT study), Urinary N-terminal cross-linked telopeptide of type 1 collagen (uNTX) and serum C-terminal cross-linked telopeptide of type 1 collagen (sCTX) levels were assessed at baseline and weeks 10 and 22 of treatment with risedronate 5mg/day. In 2302 women, responses beyond LSC in BTMs (uNTX and sCTX) and BMD (spine only) were associated with a reduced risk of nonvertebral fractures (NVFs) and all fractures. The incidence of NVF was about 50% lower in patients with reductions of uNTX of 30% or more at 22 weeks (1.6%) than in those with less than 30% reduction (3.2%) ($p = 0.015$).

Chen P et al studied changes in five BTMs in a subset of women who received daily teriparatide therapy for postmenopausal osteoporosis enrolled in the

Fracture Prevention Trial. Significant correlation was found between LS BMD response and BTMs with correlation coefficients of 0.41 for PINP, 0.40 for NTX, 0.36 for PICP, 0.28 for bone ALP and 0.23 for DPD. Amongst these, PICP increase at 1 month and PINP at 3 months correlated best with increases in LS BMD at 18 months (0.65 and 0.61, respectively; $p < 0.05$). [81]

Changes in BTMs have also been seen with other antiresorptive medications like raloxifene and strontium.[82,83]

The strong association of bone turnover markers seen with fracture risk reduction in various studies (in subjects who had been on osteoporosis treatment) supports the use of BTMs in the management of osteoporosis.

Limitations of BTMs

- Pre analytical and analytical variability
- Inadequate appreciation of sources of variability of each bone turnover markers
- Lack of standardisation of the assays for bone turnover markers
- Ethnic variations of BTMs and lack of ethnicity based reference interval for each population
- Nonavailability of data on response of various BTMs to different osteoporosis treatment and comparison between them.

Ehinicity specific reference range for BTMs

BTMs are subjected to various pre-analytical variations. Studies have shown ethnicity based variations in the distribution of BTMS. Lack of availability of reference range of BTMs in different populations and lack of standardisation of reference ranges reported by commercial labs in terms of pre-analytical variations like age , gender, ethinicity, excercise, oestrogen treatment, diurnal and seasonal variations that are known to affect BTMs measurement have necessitated the establishment of ethnicity specific reference ranges for BTMs.[84,85]

Primary prevention of osteoporosis

Osteoporosis being considered as a major public health problem, attention and measures for preventing fractures are needed at a primary health care level. Creating awareness among the community regarding the bone health is of utmost importance. Life style behavior pertaining to adequate calcium, vitamin D and protein intake, physical activity and other risk factors like smoking and alcohol needs to be addressed across all age groups for the attainment of peak bone mass and prevention of bone loss. Calcium supplementation in pre-pubertal children to the recommended daily level results in increased rate of BMD increments.[86]

Calcium with or without vitamin D supplementation has been shown to be associated with a reduction in bone loss of 0.54% at hip and 1.19% at spine and 24% reduction in the risk of fracture of all types.[87]

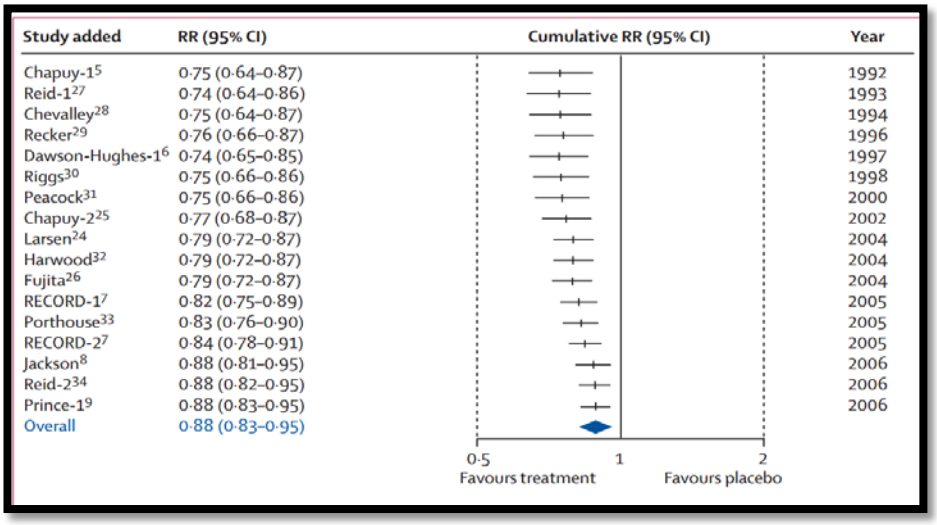


Figure-7: Meta- analysis showing the effect of calcium and calcium with vitamin D combination on the fracture risk.[87]

Physical activity is an important element which positively influences bone health, as men and women who were physically active were found to have a higher BMD and lower fracture rates compared to their sedentary counterparts.[88]

Encouraging people of all age group to be physically active not only improves the bone health by achieving a higher peak bone mass and preventing bone loss it also improves the muscle strength and postural balance leading to reduction in falls in elderly.[40,42]

Cessation of smoking and chronic alcohol consumption also improves bone health. Screening for osteoporosis using various tools for assessing low BMD, most accepted being DXA scan identify subjects with osteoporosis will help in treating these patients for preventing osteoporotic fractures. Application of FRAX:web based tools which estimate the 10 year probability of sustaining a major osteoporotic fracture and hip fractures based various risk factor assessment helps in identify at risk subjects who needs intervention. Antiresorptive and anabolic medication which target bone resorption and bone formation are available for treatment of osteoporosis.

Secondary prevention of osteoporosis

Up to half of the hip fractures are secondary to potentially modifiable risk factors like low calcium and vitamin D, low BMI, low physical activity, lower sunlight exposure and higher consumption of tea.[89]

Addressing Life style factors by improving nutrition, promoting physical activity, ensuring sunlight exposure and cessation of smoking and alcohol would prevent a second fracture. Fall preventive measures are shown intervention reducing the risk of fracture in elderly. Vitamin D supplementation may reduce the risk of fall by 19%.[90]

Several group of drugs either preventing bone resorption or promoting bone formation or both are available which are used in the treatment of osteoporosis.

Bisphosphonates are the most widely used agents for the treatment of osteoporosis which are efficacious, safe and easy to use. Upto 70% reduction of vertebral fractures, 41% reduction in hip fracture and 25% non-vertebral fracture reduction have been seen with potent bisphosphonates like Zolendronic acid.[91] Teriparatide and denosumab are the other most effective agents for preventing the fragility fractures with small differences among the drugs in terms of efficacy.[92] Monitoring the response to anti-resorptive or anabolic agents is usually by repeating a DXA scan after 18-24 months (taking into consideration of duration for the Least Significant Change to occur) to look at the increment in BMD. However, changes in BTMs are seen as early as 8-12 weeks following initiation of treatment, thus can be utilized more commonly to see the therapeutic response.

Materials & Methods

Study design

This was a cross sectional study conducted over a period of one year. Institutional review board, IRB Min.No.8343 (**OBSERVE** dated 18.06.2013) approval was obtained. Healthy premenopausal women and their mothers who had attained menopause were recruited from the community from an urban area in Vellore district of South India. Daughters and their mothers were recruited as they would be expected to have similar environmental and genetic background. Subjects were recruited from the community by random cluster sampling after obtaining an informed consent.

Inclusion Criteria

- **Premenopausal group :**

Healthy women between 25 and 45 years with cyclic monthly menses (cycles occurring every 25 to 35 days within the past year).

- **Postmenopausal group:**

Mothers of the pre-menopausal women aged above 45 years who attained menopause (Cessation of menses for a minimal period of 12 months).

Exclusion criteria

1. Systemic illnesses
2. Hyperthyroidism
3. Hyperparathyroidism
4. Cushing syndrome
5. History of fracture
6. Immobilisation
7. Either of postmenopausal women or their daughters residing outside the Vellore district.
8. Women who were on medications that may interfere with bone mineral metabolism.

These subjects were asked a detailed history regarding any physical ailments or medication use and underwent a detailed physical examination, including anthropometry.

The history included questions to assess their age, socioeconomic status, physical activity, menstrual cycles, parity, any systemic illness and medication use.

Socioeconomic status was assessed using **"Modified Kuppuswamy's socioeconomic scale",** which takes into account education, occupation and family income per month).[93] The components and categories of "Modified Kuppuswamy's socioeconomic scale" are shown in Figure-8.

(A)	Education	Score
1.	Profession or honours	7
2.	Graduate or post graduate	6
3.	Intermediate or post high school diploma	5
4.	High school certificate	4
5.	Middle school certificate	3
6.	Primary school certificate	2
7.	Illiterate	1
(B)	Occupation	Score
1.	Profession	10
2.	Semi-profession	6
3.	Clerical, shop-owner, farmer	5
4.	Skilled worker	4
5.	Semi-skilled worker	3
6.	Unskilled worker	2
7.	Unemployed	1
(C)	Family income per month	Score
1.	=30375	12
2.	15188 – 30374	10
3.	11362-15187	6
4.	7594-11361	4
5.	4556-7593	3
6.	1521-4555	2
7.	=1520	1
Total score		**Socioeconomic class**
26-29		Upper(I)
16-25	Middle	Upper middle (II)
11-15		Lower middle (III)
5-10	Lower	Upper lower (IV)
<5		Lower(V)

Figure-8: "Modified Kuppuswamy's socioeconomic scale" **(**Kumar N et al. Indian J Public Health. 2012; 56(1):103-4).

Physical activity was assessed using a internationally validated standardized questionnaire **"International Physical Activity Questionnaire" (IPAQ)**

IPAQ comprises a set of questionnaires on 4 different domains of physical actvity:

I. Work
II. Active transportation
III. Domestic or gardening
IV. Leisure time

IPAQ scoring[43]: Physical activity as measured by IPAQ could be expressed either as continuous Variable or categorical score**:**

Total Physical Activity MET-minutes/week = Total MET-minutes/week (at Work + for Transport + in Chores + in Leisure)

Categorical Score- three levels of physical activity are proposed

1. **Low**

   No activity is reported **OR**
   a. Some activity is reported but not enough to meet Categories 2 or 3.

2. **Moderate**

   Either of the following 3 criteria
   a. 3 or more days of vigorous-intensity activity of at least 20 minutes per day **OR**
   b. 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day **OR**
   c. 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of at least 600 MET-min/week.

3. **High**

   Any one of the following 2 criteria
   - Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week **OR**
   - 7 or more days of any combination of walking, moderate- or vigorous- intensity activities accumulating at least 3000 MET-minutes/week

Figure-9: Physical activity assessment using IPAQ (*Craig CL et al. Med Sci Sports Exerc. 2003 Aug; 35(8):1381-95).*

The dietary calcium intake was assessed by using a semi-quantitative food frequency questionnaire,[94] which included typical daily meal plan recall for a week. Published data on individual nutrient composition of Indian food was utilized for estimating dietary calcium intake.[95] Data was also collected regarding duration of sunlight exposure.

Subjects were examined on the day of BMD assessment. Weight was measured using same weighing machine throughout the study and height was measured by **Harpenden** stadiometer (UK). Body weighing machine and stadiometer were calibrated on a weekly basis.

Following investigations carried out on these subjects:

Biochemistry:
Albumin corrected calcium
Phosphorus
Alkaline phosphatase
Creatinine
Intact Paratharmone (iPTH)
25(OH) Vitamin-D

Bone mineral density
(Hologic DXA QDR 4500 Discovery A)
Spine(L1-L4) , femur neck, distal forearm

Bone turnover markers (BTMs)
Bone resorption markers:
Serum C-Terminal Telopeptides Type I Collagen **(CTX)**
Urine DeoxyPyridinoline**(DPD*)***
Bone formation markers:
Serum Osteocalcin **(OCN)**
Serum Procollagen Type 1N-terminal propeptide **(P1NP)**

Biochemistry:

Blood samples were collected for assessing the following **biochemical parameters** after an overnight fast: albumin corrected calcium, phosphorus, alkaline phosphatase, creatinine, paratharmone (PTH), 25(OH) vitamin D (vitamin D).

Biochemical parameters measurement was carried out in a fully automated computerized microanalyzer **(Hitachi model 911; Boehringer Mannheim, Mannheim, Germany)**. Intact PTH and vitamin D were measured by chemiluminescence immunoassay using Immulite analyzer 2000. Vitamin D deficiency has been defined by most experts as serum levels of vitamin D level less than 20 ng/ml (50n.mol/liter) and a level of 30ng/ml or greater has been considered to indicate sufficient vitamin D and values between 20 and 30 ng/dl have been considered as indicative of vitamin D insufficiency. The intra-assay and inter-assay coefficients of variation for these assays is shown in Table-6A&6B.

Table-5: Normal Ranges of parameters measured

Parameter	Normal range	Units
Serum calcium	8.3 - 10.4	mg/dl
Serum phosphorus	2.5 - 4.6	mg/dl
Serum creatinine	0.7 - 1.4	mg/dl
Serum albumin	3.5 - 5.0	g/dl
Serum alkaline phosphatase	40 - 125	u/l
Plasma PTH	8 - 50	pg/ml
25 Hydroxy-vitamin D	30 - 75	ng/ml

Precision:

Intra and interassay precision were checked in 2 control samples (15 times in a batch) and also on 30 different days. Statistical parameters are presented in **Tables (6A & 6B).**

Table 6A: Inter assay Precision:

S.No	Parameters	Mean	SD	CV %
1.	**Ca (mg/dl)**	**9.74**	**0.11**	**1.16**
2.	**Po_4 (mg/dl)**	**7.56**	**0.15**	**2.0**
3.	**Alb (g/dl)**	**1.59**	**0.04**	**2.38**
4.	**ALP(U/L)**	**51.1**	**0.7**	**1.35**
5.	**Creatinine (mg/dl)**	**2.31**	**0.04**	**1.63**

Ca – Serum Calcium PO_4 – Serum Phosphate,ALP – Serum Alkaline phosphatase, Creatinine – Serum Creatinine, Alb – Serum Albumin

Table-6B: Intra assay precision

Level 1 (n=30), Level 2 (n=30)

S.No	Parameters	Level	Mean	S. D.	CV %
1.	**Ca (mg/dl)**	1	9.0	0.1	0.9
		2	11.9	0.12	1.0
2.	**PO_4 (mg/dl)**	1	3.9	0.1	3.4
		2	7.1	0.3	4.0
3.	**Alb (g/dl)**	1	2.18	0.09	4.0
		2	4.6	0.1	2.3
4.	**ALP (U/L)**	1	87.8	1.9	2.2
		2	387	23.2	6.0
5.	**Creat (mg/dl)**	1	1.2	0.03	2.8
		2	5.2	0.1	2.5

Dual Energy X-Ray Absorptiometry (DXA):

Bone mineral density was assessed using the Discovery QDR 4500; Hologic DXA at lumbar spine, femoral neck and forearm by the same technician, which has a one pass single sweep scanning system for better quality and precision. It also eliminates errors secondary to beam overlap and image distortion which are found in rectilinear acquisition techniques, thus results in better image quality and data stability. It has a multi-element digital detector array which is paired with true fanbeam acquisition geometry, thus enabling rapid dual-energy bone mineral density measurements. DXA BMD Precision was 2 percent at both the measured sites (spine, forearm and neck of femur).

WHO classification of osteoporosis based on BMD was used for catergorisation.

Osteoporosis T score : ≤ -2.5,

Osteopenia T score : -1 to -2.4

Normal T score : > -1.

For premenopausal women low bone mass was defined as a Z score less than minus 2 at any one of the measured sites.[27]

Bone turnover markers:

Fasting blood and second morning-void urine were obtained for measurement of serum CTX, serum PTNP1, serum osteocalcin and urine pyridinoline respectively. All the blood and urine samples were collected between 8 am and 10 am after an overnight fast.

Samples of venous blood were taken from study subjects into serum separator tubes. At room temperature the blood was allowed to clot for duration of 30 min and centrifuged at 2500 rpm for 10 min at 4°C. Samples were stored at −80°C until assay. Second void urine samples were collected and stored at −20°C until assay was performed. The details of immunoassays utilised for studied Bone turn over markers (BTMs) assays are provided in Table-7.

Table-7: Bone turnover assays

BTM	Assay used	Analyser	CV%	Detection range
CTX	**Electrochemiluminescence immunoassay (ECLIA)**	**Roche elecsys Modular E170**	**4.5**	**10- 6000pg/ml**
P1NP	**Electrochemiluminescence immunoassay (ECLIA)**	**Roche elecsys Modular E170**	**2.3**	**5-1200ng/ml**
OCN	**Electrochemiluminescence immunoassay (ECLIA)**	**Roche elecsys 1010/2010**	**3.8**	**0.5-300ng/ml**
Urine DPD (Pyrilinks D)	**Chemiluminescence immunoassay (CLIA)**	**Immulite 2000**	**11**	**7- 300 nmoL/L**

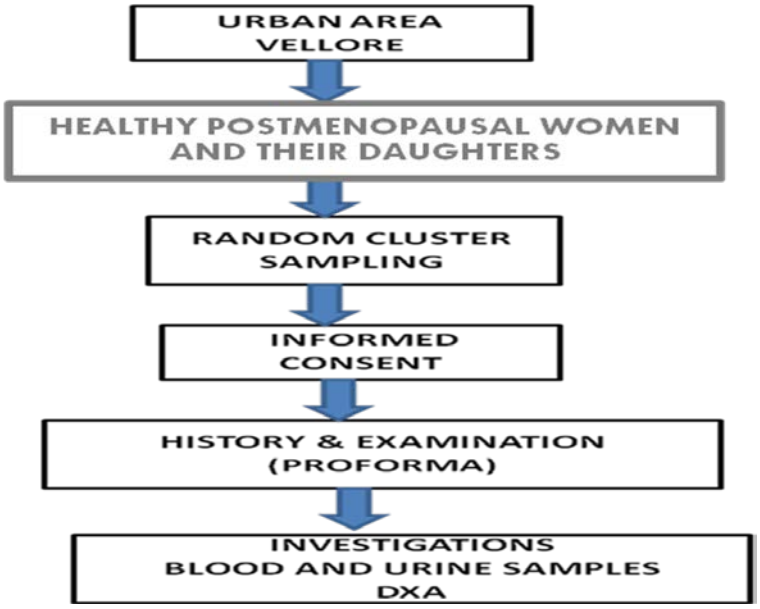


Figure-10A: Flowchart showing the recruitment protocol

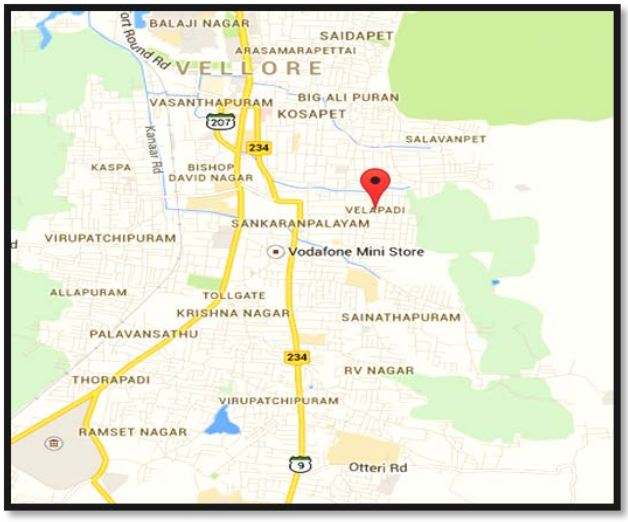


Figure-10B: Recruitment area – Velapadi area of Vellore District, Tamil Nadu, India

www.google.co.in/maps/place/Velapadi+Vellore+Tamil+Nadu+632001/@12.8984527,79.1402705,15z/data

SAMPLE SIZE CALCULATION AND STATISTICAL ANALYSIS

Sample size calculation:

A sample size of 153 is required to establish a correlation coefficient of 0.20 between FN BMD and CTX with a power of 80% and level of significance at 5%.

Statistical analysis:

The data was expressed as means or geometric mean with 95% confidence interval and categorical variables were taken when appropriate. As the values of BTMs were not equally distributed they were log transformed before analysis. For each BTM, a premenopausal and postmenopausal reference range was established by calculating the 95% reference interval of log transformed values. Levels of BTMs were compared between premenopausal and post menopausal subgroups using the student T-test, differences were considered significant at $P<0.05$.

Independent T-test was used to compare the means of two continuous variables which were normally distributed and nonparametric tests were used if their distribution was not normal. A correlation between 2 continuous variables was done using Pearson's and spearman's correlation based on the distribution of the variables. Receiver operating characteristics (ROC) curve was constructed using different cut-offs of BTMS and T-scores of LS spine and femoral neck BMD in a cohort of postmenopausal women , to derive the cutoffs for each BTMs which could best predict the risk of osteoporosis (T-score $\leq$- 2.5).

Statistical analysis was done using the SPSS 11 software package. An univariate regression analysis was carried out to assess the effect of individual factors on BMD. The factors which emerged as significant (P value ≤ 0.10) in univariate analysis were further analysed by multiple logistic regression analysis and variables were considered to be significant when P value was ≤ 0.05.

Results

This cross sectional study was conducted at an urban area in Vellore district. Two hundred subjects comprising of 100 postmenopausal women and their premenopausal daughters (n=100) were recruited by random cluster sampling. Of these 200 subjects, 48 subjects (24 pairs) were excluded based on exclusion criteria in either the postmenopausal women or their daughters (2 pairs with chronic liver disease, 3 pairs with chronic renal failure, 4 pairs who were on bone metabolism effecting medications that is 2 on steroids, one on antiepileptic Carbamazepine and one on Risperidone.

One hundred fifty two subjects which included 76 premenopausal women and their mothers (n=76) who met the inclusion criteria were recruited from the community after obtaining a detailed written informed consent.

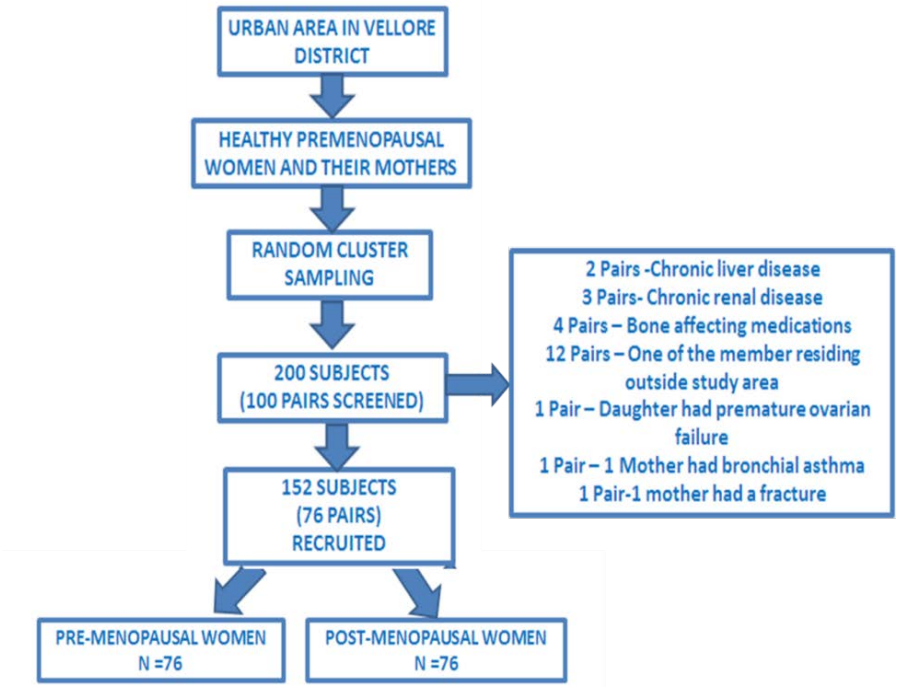


Figure-11: Recruitment of study subjects

Baseline characteristics:

Demography and other baseline characteristics are shown in Table-8. The mean ages (SD) of the premenopausal and postmenopausal group were 35.6±5.4 years and 59.0± 5.4 respectively. There was no significant difference between the BMI of post menopausal and premenopausal groups (P = NS).

Table-8: Demographic parameters

Variables	Postmenopausal Women (N=76)	Premenopausal Women (N=76)	P value
	Mean ± SD	Mean ± SD	
Age (years)	59.0± 5.4	35.6±5.4	< 0.001
BMI (kg/m^2)	24.8±4.4	25.7±4.7	NS
Parity	3.6± 1.6	2.7±1.2	NS
Dietary calcium intake (mg/24 hrs)	591±95	600±78	NS
Sunlight exposure (min/day)	118±92	107±83	NS
Physical activity (METS/week)	3335±2837	4908±3077	0.0013
Menopausal age(yrs)	45.5±4.6	-	-
Age since menopause	13.5±7.4	-	-

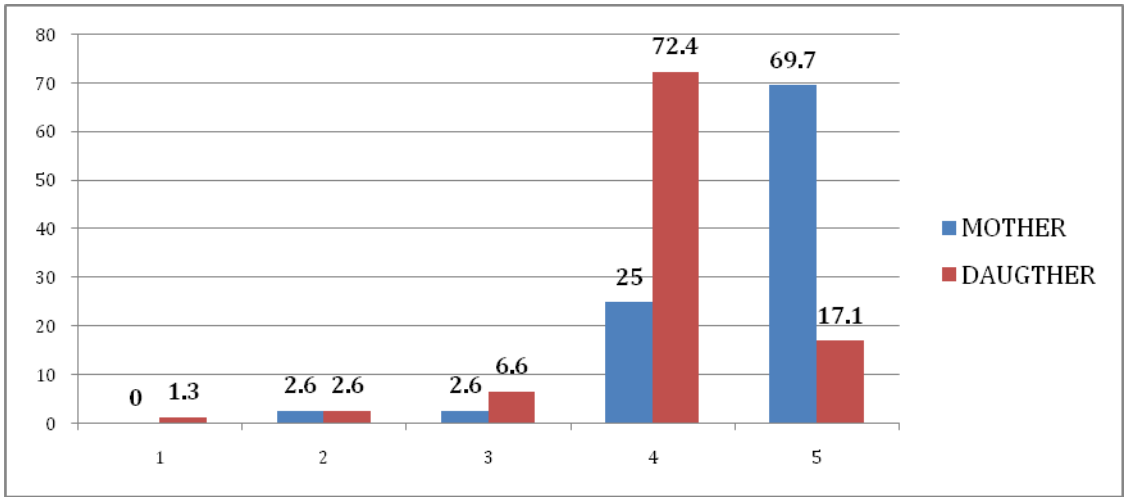
The biochemical profile of study subjects is shown in Table-9. The biochemical parameters were almost similar in both groups except for total alkaline phosphatase which was significantly higher in postmenopausal women (P <0.01). The prevalence of vitamin D deficiency (<20ng/ml) was 30% overall with 26 (34%) in premenopausal and 23 (30%) in postmenopausal groups.

Table-9: Baseline characteristics of premenopausal and postmenopausal women.

Baseline Characteristics	Postmenopausal women(N=76)	Premenopausal Women(N=76)	P value
	Mean ± SD	Mean ± SD	
Calcium (mg/dL)	9.04±0.6	8.8±0.8	NS
Albumin (gm/dL)	4.4±0.6	4.5±0.9	NS
Vitamin D (ng/ml)	24.4±9.6	23±7.6	NS
ALP (U/L)	96.5±23	72.7±16	< 0.001
Phosphorus(mg/dL)	3.9±0.4	3.6± 0.4	NS
Creatinine (mg/dL)	0.8± 0.1	0.8 ± 0.09	NS
PTH (pg/ml)	49.7± 21.8	46.2 ± 20.1	NS

Socioeconomic status

Socioeconomic status assessment using the Modified Kuppuswamy classification categorized more than two third of women in premenopausal group in upper lower class (72%) and postmenopausal women in lower class (70%).



	High	Upper middle	Lower middle	Upper lower	lower
MOTHER	0	2.6	2.6	25	69.7
DAUGTHER	1.3	2.6	6.6	72.4	17.1

Figure-12: Socioeconomic stratification of study subjects using Kuppuswamy classification

Physical Activity

Physical activity assessment using IPAQ questionnaire showed 66% (n=86) premenopausal women and 48% (n=63) postmenopausal subjects of high active physically and about 4.6% (n=7) had sedentary lifestyle all of whom were postmenopausal women.

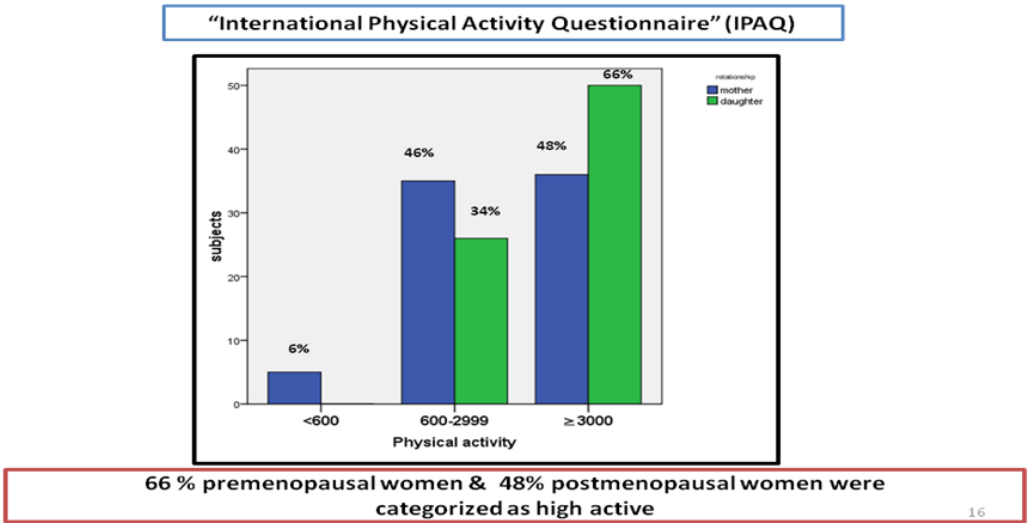


Figure 13: Categorization of physical activity using IPAQ

BMD of postmenopausal and premenopausal women

The mean BMD of both groups assessed by DXA is shown in Table-10. As expected, the mean BMD was significantly lower in postmenopausal group as compared to premenopausal group (P <0.001).

Table-10: Bone mineral density (BMD) of postmenopausal and premenopausal women

Parameters	Postmenopausal women (N=76)	Premenopausal Women (N=76)	P value
	Mean ± SD	Mean ± SD	
Lumbar spine BMD (g/cm^2)	0.791±0.144	0.984±0.112	<0.001
Femur Neck BMD (g/cm^2)	0.659±0.100	0.821±0.310	<0.001
Forearm BMD (g/cm^2)	0.460±0.082	0.634±0.663	<0.001

Prevalence of Osteoporosis & Low bone mass

In the postmenopausal group, 50% (n=38) had osteoporosis at spine and forearm and 19% (n=14) had femoral neck osteoporosis.

In the premenopausal women, low bone mass was seen in 9.2% (n=7) at spine, 18.4% (n=14) at forearm and none at femoral neck.

Table 11: **Prevalence of Osteoporosis & Low bone mass (Depicting the prevalence with and without including subjects of vitamin D deficiency)**

Site	Osteoporosis Postmenopausal women(n=76)		Low bone mass Premenopausal women (n=76)	
LS Spine	**38 (50%)**	Vit D > 20→28 (42.8%) Vit D <20 →10 (7.6%)	**7 (9.2%)**	Vit D > 20→ 2 (2.6%) Vit D < 20 →5 (6.6%)
Femur neck	**14 (19%)**	Vit D > 20→11 15.1%) Vit D < 20 →3 (3.9%)	-	
Forearm	**38 (50%)**	Vit D > 20→30 (39.4%) Vit D < 20 →8 (10.5%)	**14 (18.4%)**	Vit D > 20→11 (14.4%) Vit D < 20 →3 (3.9%)

Distribution of BTMs

The data on BTMs was not normally distributed(s expected) and hence they were log transformed to look at their reference range and log mean. After the log transformation, the data for all the BTMs showed a normal Gaussian distribution. The overall mid -95% reference range for each BTM's were derived by using geometric mean ±2 SD. Figure-14 shows the distribution of BTMs before and after log transformation.

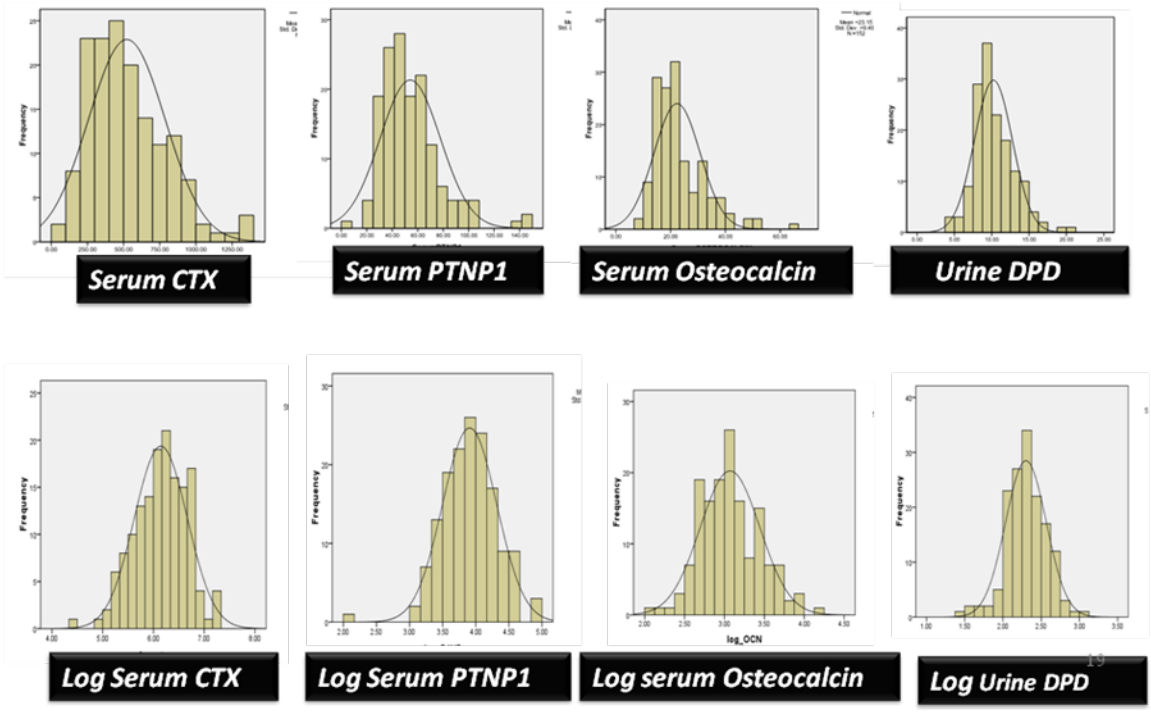


Figure 14: Distribution of BTMs before and after Log transformation

BTMs in premenopausal & postmenopausal group

Both bone resorption markers (serum CTX & Urine DPD) and Bone formation markers (OC & P1NP) were significantly higher in postmenopausal women than

premenopausal women. There was no overlap with regards to all studied BTMs among these 2 groups Figure-15.

The reference range for all BTMs was obtained by calculating the 95% reference interval of log transformed values for both premenopausal and postmenopausal women (Table-12). The reference interval for each marker is shown in (Table-13).

Table-12: Mean, Median and 95% reference range of BTMs in pre and postmenopausal women

Parameters	Postmenopausal women (N=76)			Premenopausal women (N=76)			Student T test
	Mean ± SD	Median	95% Range	Mean ± SD	Median	95% Range	P value
Serum CTX (pg/ml)	641±272.10	618	578.9 - 703.2	410±204.85	370.20	363.5-457.1	<0.001
Serum P1NP (µg/ml)	59.72±23.77	56.25	54.2 - 65.1	48.25±20.6	42.35	43.5-52.9	<0.001
Serum OC (ng/ml)	26.88±9.94	24.00	24.6-29.1	19.42±7.14	18.15	17.7-21.0	<0.001
Urine DPD (n.mol/mmol creatinine)	11.29±2.94	10.63	10.6 - 11.9	9.37±2.12	9.21	8.8-9.8	<0.001

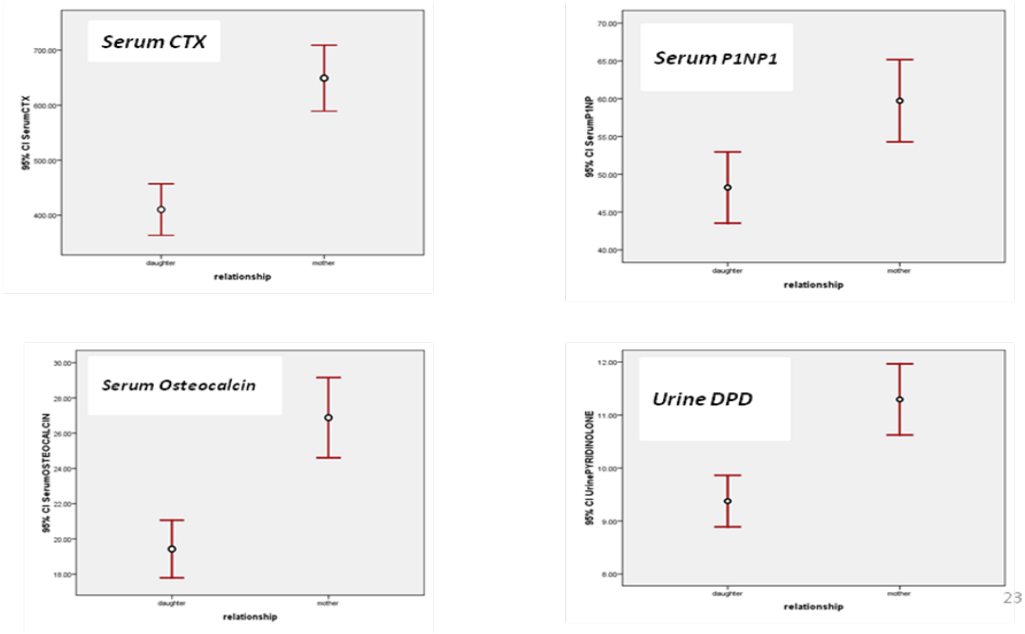


Figure-15: Graphical representation of the clear demarcation with no overlap in the reference interval of all BTMs before and after menopause.

Table-13: Reference ranges of BTMs

Parameters	Postmenopausal women	Premenopausal women
	Reference range (2.5 – 97.5 percentile)	Reference range (2.5 – 97.5 percentile)
Serum CTX (pg/ml)	**578.9 - 703.2**	**363.5 - 457.1**
Serum P1NP (ng/ml)	**54.2 - 65.1**	**43.5 - 52.9**
Serum Osteocalcin (ng/ml)	**24.6 - 29.1**	**17.7 - 21.0**
Urine DPD (n.mol/mmol creatinine)	**10.6 - 11.9**	**8.8 - 9.8**

The reference range for all bone turnover markers (which included 95% confidence interval in premenopausal and postmenopausal women) were compared with published reference range of another ethnicity. Our study population had a higher reference range of BTMs in both premenopausal and postmenopausal groups when compared to Spanish study subjects.

Table-14: Comparison of reference ranges of BTMs with published reference range from Spanish study (* Botella S, et al, J Clin Endocrinol Metab. 2013).

Parameters	Postmenopausal women	Premenopausal women
	Reference range (2.5 – 97.5 percentile)	Reference range (2.5 – 97.5 percentile)
Serum CTX (pg/ml)	**578.9 - 703.2** **420.0- 520.0***	**363.5 - 457.1** **100 -600***
Serum P1NP (ng/ml)	**54.2 - 65.1** **51.9 – 63.5 ***	**43.5 - 52.9** **20.7- 62.8 ***
Serum Osteocalcin (ng/ml)	**24.6 - 29.1** **6.36 – 8.14***	**17.7 - 21.0** **2.0- 9.9 ***
Urine DPD (n.mol/mmol creatinine)	**10.6 - 11.9** **3.8 - 13.6***	**8.8 - 9.8** **3.0- 7.4 ***

Correlation of BTM & BMD

All the 4 BTM (CTX, PTNP1, OCN and PYN) had a significant negative correlation with BMD at spine ($p<0.05$). At femoral neck, a statistically significant negative correlation of CTX, PTNP1, OCN and BMD was seen. Although there was a negative correlation between PYN & BMD, it did not attain statistical significance (Table-15) (Figure 16A, 16B, 16C & 16D).

Table-15: Correlation between BMD and BTMs

		CTX	P1NP	OC	DPD
LS BMD	r	-0.503	-0.403	-0.536	-0.277
	P	<0.001	<0.001	<0.001	<0.001
FN BMD	r	-0.208	-0.212	-0.233	-0.149
	P	0.010	0.009	0.004	0.068
FA BMD	r	-0.476	-0.348	-0.515	-0.318
	P	<0.001	<0.001	<0.001	<0.001

Correlation of CTX & spine BMD

There was significant correlation between BMD and CTX with r= -0.50, P < 0.01

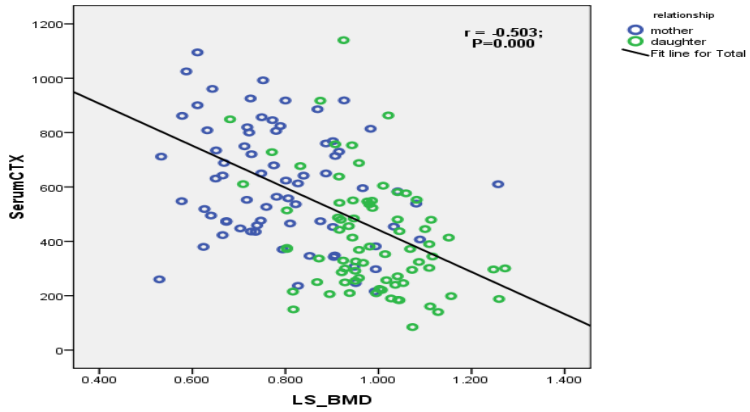


Figure-16A: Correlation of CTX & Spine BMD

Correlation of P1NP & spine BMD

There was significant correlation between BMD and P1NP with r= -0.40, P < 0.01

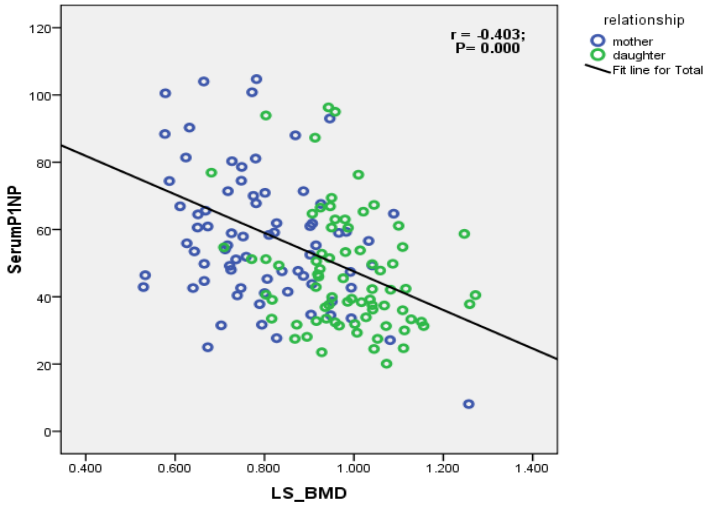


Figure-16B: Correlation of P1NP & Spine BMD

Correlation of OC & spine BMD

There was significant correlation between BMD and OC with r= -0.53, P < 0.01

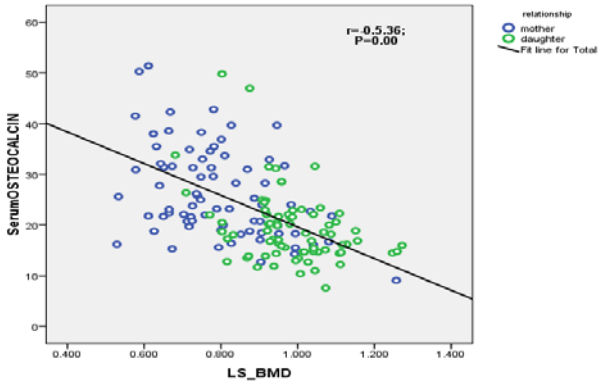


Figure-16C: Correlation of OC & Spine BMD

Correlation of DPD & spine BMD

There was a negative correlation between BMD and urine DPD but was not statistically significant(r= -0.50, P < 0.01)

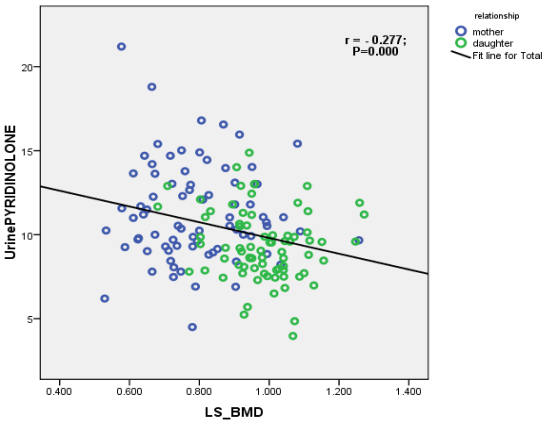


Figure-16D: Correlation of urine DPD & Spine BMD

Comparison of the BTM'S with status of bone mineral density

Serum CTX, P1NP and OC were significantly higher in women with osteoporosis as compared those women who did not have osteoporosis among the postmenopausal women (P=0.05). Urine DPD was also higher in postmenopausal women, but did not meet statistical significance (P=0.48) (Table 16).

Table-16: **Comparison of the BTMs with the status of Bone Mineral Density**

LSBMD_TSCORE		N	Mean	S D	P value
Serum CTX (pg/ml)	osteoporosis	38	702.21	254.03	**.050**
	No osteoporosis	38	585.97	256.94	
Serum P1NP (µg/ml)	osteoporosis	38	66.28	26.14	**.016**
	No osteoporosis	38	53.28	19.27	
Serum OC (ng/ml)	osteoporosis	38	30.07	10.53	**.005**
	No osteoporosis	38	23.77	8.25	
Urine DPD (n.mol/L)	osteoporosis	38	11.50	3.11	.498
	No osteoporosis	38	11.04	2.79	

Predictive value of BTMs to diagnose osteoporosis at LS spine:

The analytical performance of each BTM for the diagnosis of osteoporosis at spine by BMD assessment was studied by using Receiver Operating Curve (ROC) analysis (Figure-17A) in the postmenopausal women. All the BTMs showed a statistically significant area under the curve (AUCs) (Table 17A). The cut-off values of BTMs for prediction of osteoporosis obtained were 418pg/ml for sCTX, 46.3 µg/ml for sP1NP 19. 3 ng/ml for OC, 9.6 n.mol/L for urine DPD. (Table-17B)

ROC curve of BTMs in relation to LS BMD T-score

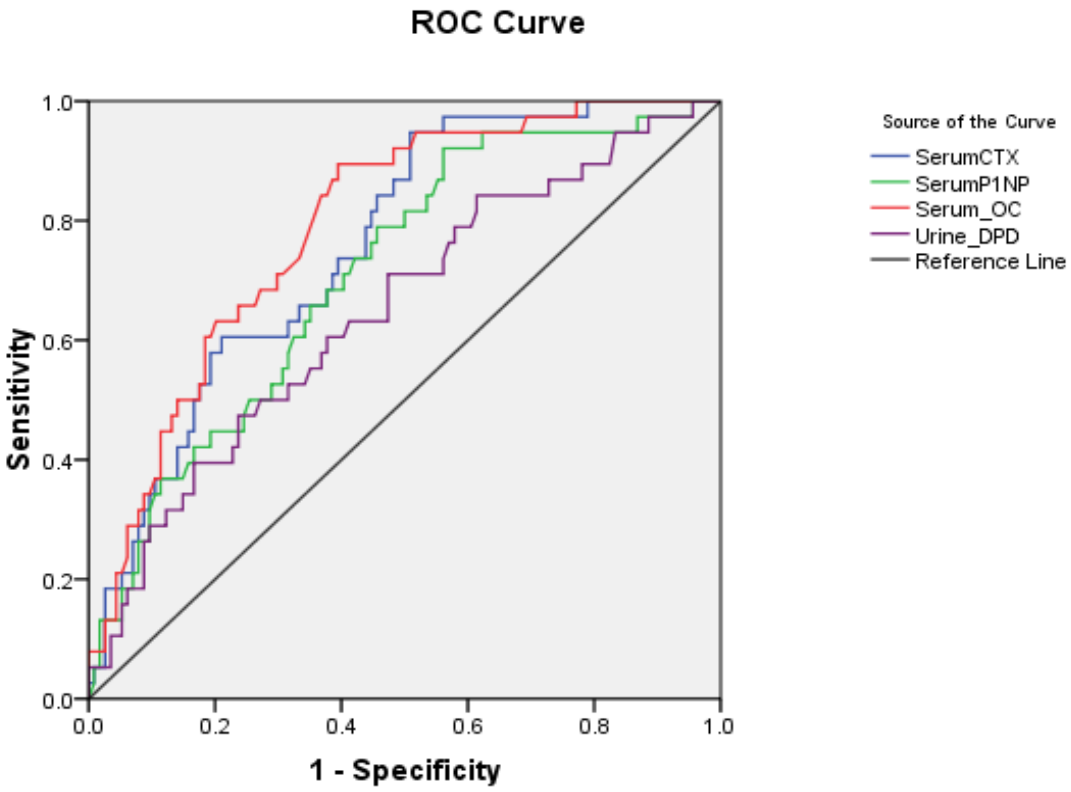


Figure-17A: ROC curve of BTMs in relation to LS BMD T-score

Table-17A: Area under curve for BTMs for predicting osteoporosis at spine

Area under curve of BTMs in predicting osteoporosis			
Variable	**Area**	**P value**	**95% CI**
SerumCTX	0.75	< 0.001	0.67 - 0.83
SerumP1NP	0.71	< 0.001	0.62 – 0.80
Serum_OC	0.79	< 0.001	0.71 - 0.86
Urine_DPD	0.64	0.006	0.54 – 0.75

Table-17B: BTMs cut-offs in predicting osteoporosis at spine

BTMs	BTM cut-off value	Sensitivity (%)	Specificity (%)
Serum CTX (pg/ml)	**418**	**94**	**50**
Serum P1NP (µg/ml)	**46.3**	**81**	**51**
Serum OC (ng/ml)	**19.3**	**94**	**50**
Urine DPD (n.mol/L)	**9.9**	**71**	**51**

Predictive value of BTMs to diagnose osteoporosis at femoral neck:

The analytical performance of each BTM for the diagnosis of osteoporosis at femoral neck by BMD assessment was studied by using Receiver Operating Curve (ROC) analysis (Figure 17B) in the postmenopausal women. All the BTMs showed a statistically significant area under the curve (AUCs) (Table-18A). The cut-off values of BTMs for prediction of osteoporosis obtained were 498pg/ml for sCTX, 49.2 µg/ml for sP1NP 21. 7 ng/ml for OC, 9.6 n.mol/L for urine DPD. (Table-18B)

ROC curve of BTMs in relation to FN BMD T-score

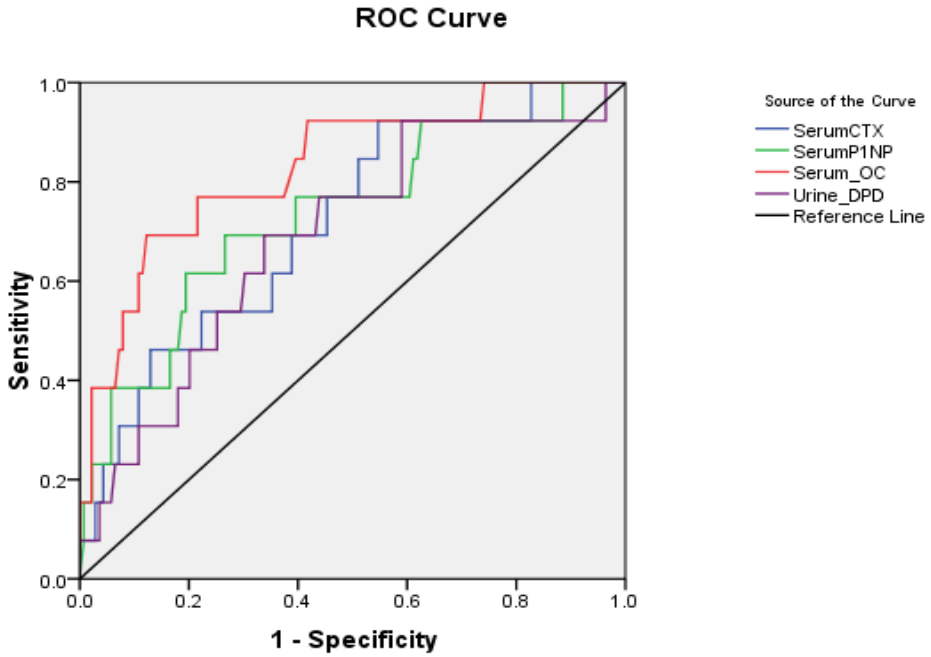


Figure 17B: ROC curve of BTMs in relation to FN BMD T-score

Table 18A: Area under curve for BTMs for predicting osteoporosis at femoral neck

Area under curve of BTMs in predicting osteoporosis			
Variable	**Area**	**P value**	**95% CI**
SerumCTX	0.71	0.010	0.57 - 0.85
SerumP1NP	0.73	0.005	0.58 – 0.88
Serum_OC	0.83	< 0.001	0.71 - 0.95
Urine_DPD	0.68	0.025	0.53 – 0.83

Table18B: BTMs cut-offs in predicting osteoporosis

BTMs	BTM cut-off value	Sensitivity (%)	Specificity (%)
Serum CTX (pg/ml)	468	84	50
Serum P1NP (µg/ml)	49.2	77	50
Serum OC (ng/ml)	21.7	92	59
Urine DPD (n.mol/L)	9.9	76	56

Comparison of the BMD of Daughters with osteoporotic mothers Vs Daughters of Non-osteoporotic mothers:

BMD of the daughters with osteoporotic mothers was compared with daughters of non osteoporotic mothers. Daughters born to mothers with osteoporosis had significantly lower BMD at spine compared to those whose mothers did not have osteoporosis

Table-19: Comparison of the BMD of Daughters with osteoporotic mothers Vs Daughters of Non-osteoporotic mothers

	Mother LS T score	n	Mean gm/cm^2	SD	P value
Daughter's LS BMD	≤-2.5	38	0.957	0.110	0.047
	>-2.5	38	1.007	0.114	

Comparison of the BTMs of Daughters' with osteoporotic mothers Vs Daugthers of Non-osteoporotic mothers:

BTMs of the daughters with osteoporotic mothers were compared with daughters of non-osteoporotic mothers. There was no significant difference in between BTMs of daughters born to mothers with osteoporosis versus those whose mothers did not have osteoporosis (Table20).

Table-20: Comparison of the BTMs of Daughters' with osteoporotic mothers Vs Daugthers of Non-osteoporotic mothers

BTMs	Mothers Spine BMD	Mean	S D	Student 't' test P value
Serum CTX (pg/ml)	Osteoporosis (n=38)	286.94	242.27	.942
	No osteoporosis (n=38)	282.72	264.64	
Serum P1NP (µg/ml)	Osteoporosis (n=38)	33.65	25.75	.839
	No osteoporosis (n=38)	35.03	33.02	
Serum OC (ng/ml)	Osteoporosis (n=38)	13.47	9.57	.863
	No osteoporosis (n=38)	13.055	11.28	
Urine DPD (n.mol/L)	Osteoporosis (n=38)	6.75	4.61	.723
	No osteoporosis (n=38)	6.36	4.87	

Risk factors for osteoporosis

At lumbar spine and forearm, a high BMI and increased physical activity had a significant positive correlation with BMD, whereas advancing age and years since menopause were found to have negative correlation ($P<0.05$).

A high BMI, increased dietary calcium intake and more duration of sunlight exposure had a positive correlation, while on the contrary, increasing age and YSM had a significant negative correlation with femoral neck BMD.

Although, there was a positive correlation seen between increased physical activity and BMD at femoral neck, it failed to attain statistical significance.

There was also significant positive correlation between vitamin D levels and sunlight exposure($r=0.25$, $P<0.001$).However, there was no correlation found between vitamin D levels and BMD at any of the 3 sites.

Table 21: Correlation between various factors influencing bone health and BMD

		LS BMD	FN BMD	FA BMD
Age	**r**	**-.624**	**-.623**	**-.598**
	P	**<.001**	**<.001**	**<.001**
BMI	**r**	**.302**	**.334**	**.299**
	P	**<.001**	**<.001**	**<.001**
Dietary Calcium	**r**	**.084**	**.210**	**.051**
	P	**.302**	**.048**	**.529**
Physical activity	**r**	**.163**	**.206**	**.275**
	P	**.045**	**.056**	**.001**
Sunlight exposure	**r**	**.163**	**.115**	**.010**
	P	**.257**	**.030**	**.388**
Age since Menopause*	**r**	**-0.280**	**-0.273**	**-0.379**
	P	**0.01 4**	**0.017**	**0.001**
Vitamin D	**r**	**-0.021**	**0.012**	**-0.101**
	P	**0.521**	**0.670**	**0.222**

*Applicable to only postmenopausal group

Association between the risk factors for osteoporosis and BTMs

There was a positive correlation between CTX, PINP, OC and Urine DPD with age. A high BMI had a significant negative correlation with CTX and OC. There was a significant negative correlation between OC & urine DPD and physical activity but did not attain statistical significance with CTX & P1NP.

Table-22: Association between the risk factors for osteoporosis and BTMs

Parameters		Serum CTX	Serum P1NP	Serum Osteocalcin	Urine Pyridinoline
Age	r	.400	.253	.392	.323
	p	<.001	.002	<.001	<.001
BMI	r	-.187	-.148	-.209	.124
	p	.021	.069	.010	.128
Dietary Calcium	r	.102	.113	.026	.079
	p	.211	.167	.750	.335
Physical activity	r	-.072	-.131	-.164	-.244
	p	.378	.108	.04	.002
Socioeconomic status	r	.359	.242	.386	.109
	p	<.001	.003	<.001	.182
Sunlight exposure	r	.079	.021	.011	-.134
	p	.336	.797	.889	.100
Age since Menopause*	r	-.069	.099	.078	.104
	p	.551	.393	.502	.372

*Applicable to only postmenopausal group

Factors affecting BMD

On multivariate analysis, low BMI and low socioeconomic status were, significantly associated with low BMD at forearm. and only LSES has emerged as a significant risk factor for less than normal BMD at lumbar spine (Table 23).

Table 23: Multivariate analysis of factors affecting BMD:

Risk factors	Lumbar spine			Forearm		
	Odds Ratio	C.I	P	Odds Ratio	C.I	P
Age (>50 yrs)	2.04	0.8 – 5.1	NS	0.66	0.1 – 3.2	NS
BMI (<25 kg/m^2)	0.66	0.2-1.7	NS	4.41	1.1 – 16.5	0.02
Parity (>2 birth)	0.54	0.1 - 1.6	NS	1.14	0.2 – 4.4	NS
Physical Activity (<3000 METs/day)	1.40	0.5 – 3.7	NS	2.27	0.7 – 7.2	NS
Dietary Calcium (<500mg/24hrs)	3.19	0	NS	0	0	NS
Socioeconomic (Low status)	3.2	1.1-16.4	0.03	5.78	1.1 – 29.4	0.03
Vitamin D (<20 ng/ml)	0.68	0.2 - 2.1	NS	2.25	0.5 – 9.4	NS
PTH (>50 pg/ml)	1.00	0.3 – 2.8	NS	0.42	0.1 – 1.6	NS

Discussion

This community based study was done in a unique cohort of healthy mothers and their daughters where we studied the relationship between the bone mineral density and bone turnover markers. We also compared the bone health of the daughters of the women who had osteoporosis with those whose mothers did not have the same. We also looked at the factors affecting BMD and BTMs in these subjects from the community.

Demography and baseline characteristics

One hundred and fifty two healthy postmenopausal women and their daughters who were premenopausal were studied. Two thirds of the study subjects were overweight according to WHO Asia specific criteria.[96]

Dietary calcium intake in our subjects was lower than the ICMR recommended daily allowance (RDA) but was higher than the previously published studies from our centre and other Indian studies.[1,97]

Around two thirds of premenopausal women and half of postmenopausal women had high physical activity score and only 4.6% in the postmenopausal women were sedentary. Our subjects were physically active either in the form of

household work both indoors and outdoors and some of our subjects were doing paid jobs which required physical exertion. However, a large number of subjects grouped as highly active could be an overestimation which has also been described as a limitation in other studies which used IPAQ questionnaire.[44]

Vitamin D status

Vitamin D deficiency was seen in one third of both premenopausal and postmenopausal subjects, unlike previously published Indian studies which showed much a higher prevalence of around 50-80%.[1, 97] This is probably due to an increased awareness created among these subjects by various educational programmes conducted by local community health services.

Vitamin D levels had a significant positive correlation with duration of sunlight exposure with about half of the subjects having sunlight exposure of more than 2 hours duration, thus highlighting the role of sunlight exposure on vitamin D status.

Osteoporosis and low bone mass

About 50 %of postmenopausal women had osteoporosis at spine and forearm which is similar to previously published studies from India.[1,98] One fifth had osteoporosis at femoral neck which constitutes an important group in view of high risk of hip fracture and its associated morbidity and mortality. The high prevalence of osteoporosis seen in our study subjects is in contradiction to about 30 % prevalence reported in American and European population.[99] Considering the number of postmenopausal women in India (about 100 million), the magnitude of this

problem is huge. Among the premenopausal women, about one tenth had low bone mass at spine which is akin to a study by Harinarayan et al in this age group.[100]

BTMs in premenopausal & postmenopausal group

Postmenopausal women had higher levels of both bone formation and bone resorption markers as compared to premenopausal women in our study.

The reference range in the postmenopausal women was higher with a steep increment from premenopausal to postmenopausal women, which has also been shown in recent studies from Saudi Arabia and Japan.[85,101] This emphasises the need for the use of different reference ranges for the pre and postmenopausal women.

We also derived reference range of BTMs specific for pre and postmenopausal women representative of South Indian urban population. The reference range of sCTX, sOC and urine DPD derived in our study seems to be higher when compared to a Spanish study; however sP1NP did not show much variation.[102] The variations in reference ranges found across different populations could be secondary to differences in the assays used in individual studies, pre-analytical variations of the BTMs, ethnicity specific differences, life style, epigenetic and genetic influences on BMD and rate of bone remodeling.[84,103] This necessitates the ethnicity specific normative data as reference range to be used in clinical practice.

To reduce the pre-analytical variability, samples for BTMs were collected in the morning after an overnight fast during the same season of the year. BTMs in our study were measured by electro-chemiluminescence immunoassay which is a robust assay as compared to other immunoassays.

Significant correlation with BMD & BTM

BMD at spine, femoral neck and forearm showed a significant correlation with all the BTMs except for urine DPD at femoral neck. Similar correlations between BTMs and BMDs have also been shown in studies by Ardawi MS and Botella S.[85,102]

Significant inverse correlation found between BTMs and BMD suggests that BTMs may be used as a valuable complementary tool to BMD in management and follow up of osteoporosis, considering its lower cost and ease of estimation.

Higher BTMs in osteoporotic group

The role of BTMs in the management of osteoporosis was further validated by the finding of significantly higher CTX, OC and P1NP in those with osteoporosis at spine as compared to those without osteoporosis. Similar findings of higher level of CTX, P1NP, OC and sclerostin were seen in osteoporotic subjects in a Spanish studies.[102,104]

Women with elevated BTMs have been found with a three to five times increased rate of bone loss.[105] BTMs assays are also cost effective as each BTM measurement costs around 300 rupees per sample, which is cheaper compared to DXA scan which costs in thousands. Thus, the measurement of BTMs seems to emerge as a promising tool for predicting the risk of fragility fractures.

Changes in BTMs following initiation of treatment are rapid and can be seen as early as 3 months, which makes it a valuable tool in monitoring subjects on treatment for osteoporosis for the therapeutic efficacy and compliance.

Diagnostic performance of BTMs

Serum CTX, PINP, OCN and Urine DPD showed good analytical performance in the diagnosis of osteoporosis at spine and femoral neck with area under curve of 0.68 -0.83.

Similar analytical performance with the sensitivity and specificity of 94% and 23% for sCTX assays and 94% and 50% for P1NP assays was also seen in a spanish study.[102] Good analytical performance with higher sensitivity but a lower specificity seen in our study as well as in recent studies by Botella S et al and Navarro CL et al suggests that BTMs can be used as a screening tool in osteoporosis.[102,104]

Mother and daughter effect of BMD and BTMs

Daughters born to mothers with osteoporosis at the spine had a significantly lower BMD at spine compared to those whose mothers did not have osteoporosis, may suggest the role of genetic, epigenetic as well as similar environmental factors in the attainment of peak bone mass. Similar findings were seen in a study from China, where daughters of mothers with osteoporosis had a lower peak bone mass as compared to daughters whose mothers did not have osteoporosis.[106]

Previously published paired studies have also showed that the inheritance of bone mass in women is determined by peak bone mass attained and thereafter decline in the bone mass secondary to bone loss occurring especially after menopause which runs in families between mothers and daughters.[107] Maternal BMD is an important determinant of bone mass of daughters which has been shown in the previous studies.[108] Genetic influence on osteoporosis is complex with multiple candidate genes have been implicated to exert small to moderate effects in the attainment of peak bone mass and bone loss.[12]

However, there was no significant difference in BTMs between daughters born to mothers with osteoporosis at spine compared to those whose mothers did not have osteoporosis.

Thus the difference seen in the lumbar spine BMD was probably due to a difference in peak bone mass and may not be due to variations seen in the bone remodeling between the two groups.

Risk factors for osteoporosis

a. Age

In our study, we found a significant negative correlation between advancing age and age since menopause with BMD at all sites, which are well established risk factors for osteoporosis.[88,109]

The factors contributing to decline in BMD and an increased fracture risk with aging includes: sex steroid deficiency, vitamin D deficiency, reduced bone formation due to intrinsic defects in the functioning of osteoblast, altered growth hormone -IGF axis and age associated sarcopenia.[110]

Increasing age is also an important risk factor for osteoporotic fractures independent of BMD. An increase in the prevalence of vertebral fractures from 14.7% at 50- 59 yrs to 22. 4% above the age of 70 years has been reported in a recent study from North India [111]

Menopausal transition results in accelerated bone loss which extends for 5-10 years and leads to 20-30% loss of trabecular bone and 5 -10% cortical bone loss.[112]

b. BMI

As shown in previous epidemiological studies, BMD at the spine and femoral neck increased with an increment in Body Mass Index in our subjects. Body weight has been described to have a protective effect on bone through various mechanisms like mechanical and gravitational effect on increasing load on the skeleton, stimulatory effect of leptin synthesized by adipocytes on bone formation, increase in the synthesizes of estrogen through increased aromatase activity which decreases bone resorption and promotes bone formation.[113]

There are recent controversies that even though obesity increases BMD, it does not seem to confer protection against a fragility fracture.[38] The bone marrow adipogenesis in obesity has been proposed to have a lyphotoxic effect on the bone forming osteoblast cell lines through various adipokines like leptin, adiponection, TNF α, IL- 6 etc.[114]

c. Dietary Calcium:

Dietary calcium intake showed a positive correlation at the femoral neck in our subjects. Adequate dietary calcium intake has been shown to be an important factor both for attainment of peak bone mass and maintenance of bone health.[24] Beneficial effect of calcium supplementation is probably secondary to gain in bone mass and reduction in bone remodeling.[32]

Adequate calcium and vitamin D intake are most important aspects in preventing rapid bone loss in the high risk groups like most Indian postmenopausal women and elderly men as their intake calcium and vitamin D have been shown to be low in many community based studies.[1,37]

In meta-analysis looking at the effect of calcium supplementation on risk of fracture and bone loss, a risk reduction of 12% with respect to all types of fractures (RR: 0·88, CI :0·83–0·95), bone loss reduction rate of 0.5% at hip (CI:0·35–0·73) and 1.19% at spine (CI:0·76–1·61) was seen with greater effect evident at higher compliance and doses of 1200 mg or more for calcium and 800 U or more for vitamin D.[87]

d. Physical activity

BMD at all spine, femoral neck and forearm showed a positive correlation with the physical activity in our study.

A high physical activity has been shown to result in a higher BMD and a reduced risk of fracture in previously published studies. The risk of hip fracture was shown to increase by 2 fold in those women who walked for 4 hours or less as compared to those who walked for more than 4 hours.[88]

The effect of exercise on bone health seems to be maximal during the pubertal period. Physical activity is an important determinant of osteoporosis as it is not only required for attainment and maintenance of bone mass, it also improves the muscle strength and balance thus reduces the risk of falls and fractures.[42,115]

e) Socio economic status

Low socioeconomic status (LSES) is an important risk factor for osteoporosis. Finding of an adverse impact of LSES on bone health in our study has significant implications in a developing country like India with regards to economy and planning policies to improve nutrition and social well being. Two third of our subjects were from a lower socioeconomic status. Poverty is a modifiable

environmental risk factor which affects the bone health through nutritional factors like deficiency of calcium, vitamin D and protein intake which affect both the attainment of peak bone mass and also increases the bone loss.

In a study by Navarro MC et al, women from lower socioeconomic status were found to have a higher prevalence of osteoporosis as compared to those in medium and higher socioeconomic strata[40.6% Vs 35.6%;odds ratio1.35, CI:1.03-1.76], higher fragility and vertebral fractures respectively [37.8% Vs 27.7%; odds ratio: 1.45, CI:1.11-1.90] and [24.7% vs 13.4%;odds ratio:2.01, CI:1.44-2.81].[45]

Indian women from lower income group have been shown to have a lower bone mineral density secondary to nutritional factors like low dietary calcium intake.[116]

Factors affecting BTM

There was an increase in both formation and resorption markers with advancing age, thus pointing towards an augmented bone remodelling and increased bone loss with age. In a study from Saudi Arabia, the bone formation markers showed a declining trend till the age of 49, after which there was a steep increase in the age group of 50-59 years. Bone resorption markers showed a similar trend with decrease till 44 years of age, followed by steep increase.[85] Similarly, there was a sharp increase in BTMs from premenopausal period to postmenopausal age group in our study.

There was decrease in bone remodeling and bone loss with increasing body weight and higher physical activity as evidenced by a negative correlation seen between bone turnover markers and BMI as well as physical activity in the present study. Significantly lower serum CTX, urinary NTX and CTX and a lower serum

OC and P1NP were seen with an increase in BMI, which is also been shown in Saudi Arabian population.[85]

Strengths of the study

This is the first community based cross sectional study to look at the correlation between various BTMs and BMD and the impact of maternal bone mineral status on bone health of daughters in a cohort of premenopausal women and their mothers recruited by the cluster sampling technique. The reference range for various BTMs was derived. This study also looked at the several factors including physical activity as well as socio economic status and their influence on BMD and BTMs.

Limitations of the study

1) This study was done in an urban area and may not represent rural community.
2) The sample size was calculated to study the correlation between BTMs and BMD. The reference range generated for BTMs will thus need further validation in a larger cohort of subjects.
3) In premenopausal women, collection of blood or urine sample for BTMs was regardless of particular phase of menstrual cycle.

Conclusion

A total number of one hundred fifty two healthy women which included 76 postmenopausal women and their daughters (n=76) from the community were enrolled in this study over a period of 1 year (2013-2014).

The following conclusions were drawn at the end of analysis.

1. Fifty percent of the postmenopausal women had osteoporosis at spine and forearm and femoral neck osteoporosis was seen in one fifth, which constitutes an important group in view of high risk of sustaining a hip fracture. Among the premenopausal women, about one tenth had low bone mass at spine.

2. Postmenopausal women had higher bone turnover markers including both bone formation and bone resorption markers as compared to the premenopausal women. Ethnicity based reference range of BTMs serum C-Terminal Telopeptides Type I Collagen (CTX), serum Procollagen type I N-terminal propeptide (P1NP), serum Osteocalcin (OC) and urine Deoxy Pyridinoline (DPD) were studied.

3. Significant inverse correlation was found between all the measured BTMs and BMD with significantly higher BTMs levels in osteoporotic subjects suggesting that BTMs may be used as a complementary tool to BMD in management and follow up of osteoporosis, considering their lower cost and ease of estimation.

4. Daughters born to mothers with osteoporosis at spine had significantly lower BMD at spine compared to those whose mothers did not have osteoporosis, suggesting the role of probable interplay of genetic, epigenetic and similar environmental factors in the attainment of peak bone mass.

5. A significant inverse correlation was seen between BMD at spine, femoral neck and forearm with advancing age, years since menopause, low physical activity denoting that both modifiable and non-modifiable factors play significant role in determining the bone mass at one point of time.

6. Low socio-economic status was found to have a detrimental effect on BMD at spine (Odds 3.2, P=0.03) and forearm(Odds 5.7, P=0.03).

Recommendations

The following recommendations have been drafted based on this study.

1. Osteoporosis was seen in half of the postmenopausal women in the community and low bone mass seen in one tenth of the premenopausal women. This necessitates the importance of simple interventions to improve the peak bone mass and reduce the bone loss by optimising calcium and vitamin D intake, encouraging physical activity, awareness and allocation of resources for screening and treatment of osteoporosis in the community.

2. The reference range for various BTM's studied in a well-defined cohort of premenopausal women and their mothers differed from the reference ranges published from other ethnicities. Hence, there is a need for a study with larger sample size for nationwide standardization of BTM's measurements by commercially available chemiluminescence immunoassay methods.

3. In view of good analytical performance of BTM's in the diagnosis of osteoporosis and the cost effectiveness and ease of estimation of their assays, in future it may be possible to use BTM's as a screening tool in osteoporosis. However, it has to

be further validated in a large cohort of postmenopausal subjects with osteoporosis and age and BMI matched control women. In addition, BTM's may also provide insights to the bone quality and phases of bone remodeling.

4. Large epidemiologic studies looking at the role of BTMs in predicting the fracture risk, independent of BMD in an Indian context are required.

5. Prospective studies looking at the response of BTMs to treatment of osteoporosis with currently available anti-osteoporotic medications are needed which will substantiate their use in the follow up of these patients.

Bibliography

1. Meeta, Digumarti L, Agarwal N, Vaze N, Shah R, Malik S. Clinical practice guidelines on menopause: An executive summary and recommendations. ***J Midlife Health.*** 2013;4(2):77-106
2. Paul TV, Thomas N, Seshadri MS, Oommen R, Jose A, Mahendri NV. Prevalence of osteoporosis in ambulatory postmenopausal women from a semiurban region in Southern India: relationship to calcium nutrition and vitamin D status. ***Endocr Pract.*** 2008;14(6):665-71
3. Shetty S, Kapoor N, Naik D, Asha HS, Prabu S, Thomas N, Seshadri MS, Paul TV. Osteoporosis in healthy South Indian males and the influence of life style factors and vitamin d status on bone mineral density. ***J Osteoporos.*** 2014;1-5.
4. Mithal A, Bansal B, Kyer CS, Ebeling P. The Asia-Pacific Regional Audit-Epidemiology, Costs, and Burden of Osteoporosis in India 2013: A report of International Osteoporosis Foundation. ***Indian J Endocrinol Metab.*** 2014; 18(4):449-54.
5. Paul TV, Selvan SA, Asha HS, Thomas N, Venkatesh K, Oommen AT, Mathai T, Daniel AJ, Seshadri MS. Hypovitaminosis D and other risk factors of femoral neck fracture in South Indian Postmenopausal women-A Pilot Study. ***J Clin Diagn Res***. Forthcoming 2015
6. Handa, R. Action Plan Osteoporosis: Consensus Statement of the Expert Group Meeting, Osteoporosis Society of India. New Delhi. 2003;1–40. Available from: ***http://www.osteofound.org/press centre/fact_sheet.html.***

7. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. ***BMJ***. 1996; 312(7041):1254-9.
8. Nguyen ND, Eisman JA, Center JR, Nguyen TV. Risk factorsfor fracture in non-osteoporotic men and women. ***J Clin Endocrinol Metab.*** 2007; 92(3):955-62.
9. Mitchell BD, Yerges-Armstrong LM. The genetics of bone loss: challenges and prospects. ***J Clin Endocrinol Metab.*** 2011;96(5):1258-68.
10. Wheater G, Elshahaly M, Tuck S, Datta K H , Laar J MV . The clinical utility of bone marker measurements in osteoporosis. ***J Transl Med***. 2013;11:201.
11. Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, McClung M, Morris HA, Silverman S, TrentiT, Wahl DA, Cooper C, Kanis JA. IOF-IFCC Bone Marker Standards Working Group: Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. ***Osteoporos Int.*** 2011; 22:391–420.
12. Ralston SH.Genetic control of susceptibility to osteoporosis. ***J Clin Endocrinol Metab.*** 2002;87(6):2460-6.
13. World Health Organization. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. ***World Health Organ Tech Rep Ser.*** 1994;843:1–129

14. Gupta AK, Samuel KC, Kurian PM, Rallan RC. Preliminary study of the incidence and aetiology of femoral neck fracture in Indians. ***Indian J Med Res.*** 1967;55:1341-8.
15. Dhanwal DK, Siwach R, Dixit V, Mithal A, Jameson K, Cooper C. Incidence of hip fracture in Rohtak district. North India. ***Arch Osteoporos.*** 2013;8(1-2):135.
16. Dhanwal DK, Cooper C, Dennison EM. Geographic variation in osteoporotic hip fracture incidence: the growing importance of Asian influences in coming decades. ***J Osteoporos.*** 2010;2010:5.
17. Dhanwal DK, Sahoo S, Gautam VK, Saha R. Hip fracture patients in India have vitamin D deficiency and secondary hyperparathyroidism. ***Osteoporos Int.*** 2013;24(2):553-7.
18. Shuler FD, Conjeski J, Kendall D, Salava J. Understanding the burden of osteoporosis and use of the World Health Organization FRAX. ***Orthopedics.*** 2012;35(9):798-805.
19. Ray NF, Chan JK, Thamer M, Melton LJ. Medical expenditures for the treatment of osteoporotic fractures in the U.S. in 1995: report from the NOF. ***J Bone Miner Res***. 1997;12:24-35.
20. Col NF, Eckman MH, Karas RH, et al. Patient specific decisions about hormone replacement therapy in postmenopausal women. ***JAMA.*** 1997;227:1140-1147.
21. Chrischilles EA, Butler CD, Davis CS, et al. A model of lifetime osteoporosis impact. ***Arch Intern Med***. 1991;151:2026-2032.

22. DelmasPD.Treatment of postmenopausalosteoporosis.***Lancet***. 2002;359(9322): 2018-26.
23. Bonjour JP, Theintz G, Law F, Slosman D, Rizzoli R. Peak bone mass. ***Osteoporos Int.*** 1994;4(Suppl 1):7-13.
24. Rizzoli R. Nutritional aspects of bone health. ***Best Pract Res Clin Endocrinol Metab.*** 2014;28(6):795-808.
25. Mithal A, Kaur P. Osteoporosis in Asia: a call to action. ***Curr Osteoporos Rep.*** 2012;10(4):245-7.
26. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. ***Lancet.*** 2002;359:1929–1936.
27. World Health Organization (WHO), "Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: report of a WHO study group". ***WHO Technical Report 843, WHO, Geneva, Switzerland.*** 1994.
28. Cefalu CA. Is bone mineral density predictive of fracture risk reduction?. ***Curr Med Res Opin.*** 2004;20(3):341-9.
29. Nguyen ND, Eisman JA, Center JR, Nguyen TV. Risk factors for fracture in nonosteoporotic men and women. ***J Clin Endocrinol Metab***. 2007;92(3):955-62.
30. Holroyd C, Harvey N, Dennison E, Cooper C. Epigenetic influences in the developmental origins of osteoporosis. ***Osteoporos Int.*** 2012;23(2):401-10.
31. Lee et al.: Association between dietary calcium and phosphorus intakes, dietary calcium / phosphorus ratio and bone mass in the Korean population. ***Nutrition Journal***. 2014;13:114.

32. Bhatia V. Dietary calcium intake - a critical reappraisal. ***Indian J Med Res***. 2008;127: 269-273.
33. Chevalley T, Bonjour JP, van Rietbergen B, et al. Tracking of environmental determinants of bone structure and strength development in healthy boys: an eight-year follow up study on the positive interaction between physical activity and protein intake from prepuberty to mid-late adolesecence. ***J Bone Mineral Res***. 2014;29(10):2182-92.
34. Zhang M, Xuan S, Bouxsein ML, et al. Osteoblast-specific knockout of the insulin-like growth factor (IGF) receptor gene reveals an essential role of IGF signaling in bone matrix mineralization. ***J Biol Chem.*** 2002;277:4400.
35. Caverzasio J, Montessuit C, Bonjour JP. Stimulatory effect of insulin-like growth factor-1 on renal Pi transport and plasma 1,25-dihydroxyvitamin D3. ***Endocrinology.*** 1990;127:453.
36. Wahl DA, Cooper C, Ebeling PR, Eggersdorfer M, Hilger J, Hoffmann K, Josse R, Kanis JA, Mithal A, Pierroz DD, Stenmark J, Stöcklin E, Dawson-Hughes B. A global representation of vitamin D status in healthy populations. ***Arch Osteoporos.*** 2012;7(1-2):155-72.
37. Harinarayan CV, Joshi SR. Vitamin D status in India-its implications and remedial measures. ***J Assoc Physicians India.*** 2009;57:40-8.
38. Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, Pfeilschifter J, Silverman S, Díez-Pérez A, Lindsay R, Saag KG, Netelenbos JC, Gehlbach S, Hooven FH, Flahive J, Adachi JD, Rossini M, Lacroix AZ, Roux C, Sambrook PN, SirisES.Glow Investigators. Obesity

is not protective against fracture in postmenopausal women: GLOW. ***Am J Med***. 2011;124(11):1043–50.

39. Johansson H, Kanis JA, Odén A, McCloskey E, Chapurlat RD, Christiansen C et al. A meta-analysis of the association of fracture risk and body mass index in women. ***J Bone Miner Res.*** 2014;29(1):223-33.
40. Devine, A., Dhaliwal, S. S., Dick, I. M., Bollerslev, J. and Prince, R. L. Physical Activity and Calcium Consumption Are Important Determinants of Lower Limb Bone Mass in Older Women. ***J Bone Miner Res***. 2004;19:1634–1639.
41. Tobias JH, Gould V, Brunton L, Deere K, Rittweger J, Lipperts M, Grimm B. Physical Activity and Bone: May the Force be with you. ***Front Endocrinol (Lausanne)***. 2014;5:20.
42. Karl Karlsson M, Erik Rosengren B. Physical activity as a strategy to reduce the risk of osteoporosis and fragility fractures. ***Int J Endocrinol Metab.*** 2012;10(3):527-36.
43. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, OjaP. International physical activity questionnaire: 12-country reliability and validity. ***Med Sci Sports Exerc***. 2003;35(8):1381-95.
44. Lee PH, Macfarlane DJ, Lam T, Stewart SM. Validity of the international physical activity questionnaire short form (IPAQ-SF): A systematic review. ***International Journal of Behavioral Nutrition and Physical Activity***. 2011; 8:115.

45. Navarro MC, Sosa M, Saavedra P, Lainez P, Marrero M, Torres M, Medina CD. Poverty is a risk factor for osteoporotic fractures. ***Osteoporos Int***. 2009;20(3):393-8.

46. Brennan SL[1], Quirk SE, Hosking SM, Kotowicz MA, Holloway KL, Moloney DJ, Dobbins AG, Pasco JA. Is There an Interaction Between Socioeconomic Status and FRAX 10-Year Fracture Probability Determined With and Without Bone Density Measures? Data from the Geelong Osteoporosis Study of Female Cohort. ***Calcif Tissue Int.*** 2015;96(2):138-44.

47. Marwaha RK, SripathyG.Vitamin D & bone mineral density of healthy school children in northern India. ***Indian J Med Res.*** 2008;127(3):239-44.

48. Zhu K, Prince RL. Lifestyle and osteoporosis. ***Curr Osteoporos Rep***. 2015;13(1):52-9.

49. J. A. Kanis, O. Johnell, A. Oden, H. Johansson, and E. McCloskey, "FRAX and the assessment of fracture probability in men and women from the UK". ***Osteoporosis International***. 2008;19(4);385–397.

50. Shetty S, Kapoor N, Naik D, Asha HS, Thomas N, Paul TV. The impact of the Hologicvs the ICMR database in diagnosis of osteoporosis among South Indian subjects. ***Clin Endocrinol (Oxf).*** 2014;81(4):519-22.

51. Carey JJ, Licata AA, Delaney MF: Biochemical markers of bone turnover. ***Clin Rev Bone Miner Metab***. 2006;4(3):197–212

52. Lian JB and Stein GS. The cells of bone. In: Dynamics of bone and cartilage metabolism. ***Seibel MJ, Robins SP, Bilezikian JP (eds), Academic Press, San Diego***; 1999;165–86.

53. Rizzoli R and Bonjour JP. Physiology of calcium and phosphate homeostasis. In: Dynamics of bone and cartilage metabolism. ***Seibel MJ, Robins SP, Bilezikian JP (eds), Academic Press, San Diego***. 1999;247–60.
54. Klein-Nulend J, Bakker AD, Bacabac RG, Vatsa A, Weinbaum S: Mechanosensation and transduction in osteocytes. ***Bone.*** 2013;54(2):182–190.
55. Vega D, Maalouf NM, Sakhaee K: The role of receptor activator of nuclear factor-κB (RANK) / RANK Ligand/ Osteoprotegerin: clinical implications. ***J Clin Endocrinol Metab***. 2007;92(12):4514–4521.
56. Seibel J. Biochemical markers of bone turnover: part I: biochemistry and variability. ***Clin Biochem Rev***. 2005;26(4): 97–122.
57. Garnero, P. et al. Decreased β-isomerization of the C-terminal telopeptide of type I collagen αI chain in Paget's disease of bone. ***J Bone Miner Res.*** 1997;12:1407–1415.
58. Garnero, P. et al. Effects of PTH and alendronate on type I collagen isomerization in postmenopausal women with osteoporosis: the PaTH study. ***J Bone Miner Res.*** 2008;23(9):1442-8.
59. Hannon, R. & Eastell, R. Preanalytical variability of biochemical markers of bone turnover. ***Osteoporos Int.*** 2000;11(Suppl. 6), S30–S44.
60. Qvist, P., Christgau, S., Pedersen, B. J., Schlemmer, A. & Christiansen, C. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. ***Bone.*** 2002;31:57–61.

61. Clowes, J. A. et al. Effect of feeding on bone turnover markers and its impact on biological variability of measurements. ***Bone***. 2002;30:886–890.
62. Baxter I, Rogers A, Eastell R, Peel N: Evaluation of urinary N-telopeptide of type I collagen measurements in the management of osteoporosis in clinical practice. ***Osteoporos Int.*** 2013;24:941–947.
63. Robins, S. P. et al. Direct enzyme linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. ***J Bone Miner Res.*** 1994;1643–1649.
64. Halleen JM, Alatalo SL, Janckila AJ et al. Serum tartrateresistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. ***Clin Chem.*** 2001;47:597–600.
65. Hannon R, Blumsohn A, Naylor K et al. Response of biochemical markers of bone turnover to hormone replacement therapy: impact of biological variability. ***J Bone Miner Res.*** 1998;13:1124–1133
66. Garnero P, Vergnaud P, Hoyle N. Evaluation of a fully automated serum assay for total N-terminal propeptide of type I collagen in postmenopausal osteoporosis. ***Clin Chem.*** 2008;54:188–196.
67. Brandt J, Krogh TN, Jensen CH, Frederiksen JK, Teisner B: Thermal instability of the trimeric structure of the N-terminal propeptide of human Procollagen type I in relation to assay technology. ***Clin Chem.*** 1999;45(1):47–53.

68. Stinson RA, Hamilton BA. Human liver plasma membranes contain an enzyme activity that removes membrane anchor from alkaline phosphatase and converts it to a plasma-like form. ***Clin Biochem***. 1994;27:49–55.
69. Brown JP, Delmas PD, Malaval L, et al. Serum bone Gla-protein: a specific marker for bone formation in postmenopausal osteoporosis. ***Lancet.*** 1984;1:1091–3.
70. Vergnaud, P. et al. Undercarboxylatedosteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS study. ***J Clin. Endocrinol Metab.*** 1997;82:719–724.
71. Clemens, T. L. &Karsenty, G. The osteoblast: an insulin target cell controlling glucose homeostasis. ***J Bone Miner Res.*** 2001;26:677–680.
72. Szulc P, Douglas C. Biochemical markers of bone turnover in osteoporosis. ***The Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 8th Edition:*** 297- 302.
73. Dawson-Hughes B, Lindsay R, Khosla S, Melton LJ, Tosteson AN, Favus MJ, Baim S. Clinician's guide to prevention and treatment of osteoporosis. ***National Osteoporosis Foundation***. 2008.
74. Follet H, Boivin G, Rumelhart C, Meunier PJ. The degree of mineralization is a determinant of bone strength: a study on human calcanei. ***Bone.*** 2004;34:783–789.
75. Banse X, Sims TJ, Bailey AJ. Mechanical properties of adult vertebral cancellous bone: correlation with collagen intermolecular cross-links. ***J Bone Miner Res.*** 2002;17:1621–1628.

76. Garnero P, Sornay-RenduE, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. ***J Bone Miner Res*** .2000;15:1526–1536.

77. Van Daele PL, Seibel MJ, Burger H, Hofman A, Grobbee DE, van Leeuwen JP, Birkenhager JC, Pols HA. Case–control analysis of bone resorption markers, disability, and hip fracture risk: the Rotterdam study. ***BMJ.*** 1996;312:482–483.

78. Vergnaud P, GarneroP,Meunier PJ, Breart G, Kamihagi K, Delmas PD. Under carboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS study. ***J Clin Endocrinol Metab***.1997;82:719–724

79. Chapurlat RD, Garnero P, Bréart G, Meunier PJ, Delmas PD. Serum type I collagen breakdown product (serum CTX) predicts hip fracture risk in elderly women: the EPIDOS study. ***Bone.*** 2000;27:283–286.

80. Eastell R, Vrijens B., Cahall D. L., Ringe J. D., Garnero P. and Watts N. B. Bone turnover markers and bone mineral density response with risedronate therapy: Relationship with fracture risk and patient adherence. ***J Bone Miner Res.*** 2011;6: 662–1669.

81. Chen P, Satterwhite JH, Licata AA, Lewiecki EM, Sipos AA, Misurski DM &Wagman RB. Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis. ***J Bone Miner Res.*** 2005;20(6):962–970.

82. Hansdóttir H, Franzson L, Prestwood K, Sigurdsson G.The effect of raloxifene on markers of bone turnover in older women living in long-term care facilities. ***J Am Geriatr Soc.*** 2004;52(5):779-83.

83. Meunier PJ, Roux C, Seeman E et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. ***N Engl J Med.*** 2004;350:459– 468.

84. de Papp AE, Bone HG, Caulfield MP, Kagan R, Buinewicz A, Chen E, Rosenberg E, Reitz RE. A cross-sectional study of bone turnover markers in healthy premenopausal women. ***Bone.*** 2007;40(5):1222-30.

85. Ardawi MS[1], Maimani AA, Bahksh TA, Rouzi AA, Qari MH, Raddadi RM. Reference intervals of biochemical bone turnover markers for Saudi Arabian women: a cross-sectional study. ***Bone.*** 2010;47(4):804-14.

86. Johnston Jr CC, Miller JZ, Slemenda CW, et al. Calcium supplementation and increases in bone mineral density in children. ***N Engl J Med***. 1992;327:82.

87. Tang BM, Eslick GD, Nowson C, et al. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. ***Lancet.*** 2007;370: 657.

88. Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. ***N Engl J Med.*** 1995; 332(12):767-73.

89. Johnell O, Gullberg B, Kanis JA, Allander E, Elffors L, Dequeker J, Dilsen G, Gennari C, Lopes Vaz A, Lyritis G. Risk factors for hip

fracture in European women: the MEDOS study. Mediterranean Osteoporosis Study. ***J Bone Miner Res.*** 1995;10(11):1802-15

90. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP, Henschkowski J. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. ***BMJ.*** 2009;339:b3692.
91. Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, Cosman F, Lakatos P, Leung PC, Man Z, Mautalen C, Mesenbrink P, Hu H, Caminis J, Tong K, Rosario-Jansen T, Krasnow J, Hue TF, Sellmeyer D, Eriksen EF, Cummings SR; Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. HORIZON Pivotal Fracture Trial. ***N Engl J Med.*** 2007;356(18):1809-22.
92. Murad MH, Drake MT, Mullan RJ, et al. Clinical review. Comparative effectiveness of drug treatments to prevent fragility fractures: a systematic review and network meta-analysis. ***J Clin Endocrinol Metabol***. 2012; 97:1871.
93. Kumar N, Gupta N, Kishore J. Kuppuswamy's socioeconomic scale: updating income ranges for the year 2012. ***Indian J Public Health.*** 2012; 56(1):103-4.
94. Willett WC, Sampson L, Stampfer MJ, et al.Reproducibility and validity of a semiquantitative food frequencyquestionnaire. ***Am J Epidemiol.*** 1985;122:51-65.
95. Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N. Prevalence and significance oflow 25-hydroxyvitamin D concentrations in healthy subjectsin Delhi. ***Am J ClinNutr***. 2000; 72:472-475.

96. WHO Expert Consultation. Appropriate body mass index for Asian populations and its implications for policy and intervention strategies. ***Lancet***. 2004;363(9403):157-63.

97. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao PV, Sarma KV, Kumar EG. High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy south Indians. ***Am J Clin Nutr.*** 2007; 85(4):1062-7.

98. Marwaha R K, Tandon N, Garg MK, Kanwar R, Narang A, Sastry A, Saberwal A, Bhadra K, Mithal A. Bone health in healthy Indian population aged 50 years and above. ***Osteoporos Int***. 2011; 22(11):2829-36.

99. National Osteoporosis Foundation Releases New Data Detailing the Prevalence of Osteoporosis. Available from: ***http://nof.org/news/nofnews/2011-0510 -AdvocacyNews*** (accessed 13 April 2013).

100. Harinarayan CV, Sachan A, Reddy PA, Satish KM, Prasad UV, Srivani P. Vitamin D status and bone mineral density in women of reproductive and postmenopausal age groups: a cross-sectional study from south India. ***J Assoc Physicians India***. 2011; 59:698-704.

101. Iki M, Akiba T, Matsumoto T, Nishino H, Kagamimori S, Kagawa Y, et al. Reference database of biochemical markers of bone turnover for Japanese female population. Japanese population-based osteoporosis (JPOS) Study. ***Osteoporos Int***. 2004; 15: 981–91.

102. Botella S, Restituto P, Monreal I, Colina I, Calleja A, Varo N.Traditional and novel bone remodeling markers in premenopausal and postmenopausal women. ***J Clin Endocrinol Metab.*** 2013;98(11):E1740-8.

103. Finkelstein JS, Lee ML, Sowers M, Ettinger B, Neer RN, Kelsey JL, et al. Ethnic variation in bone density in premenopausal and early perimenopausal women: Effects of anthropometric and lifestyle factors. ***J Clin Endocrinol Metab***. 2002;87: 3057–67.

104. Navarro Casado L, Blazquez Cabrera JA, Del Pino Montes J, Almar Marques E, Chafer Rudilla M, Mateos Rodriguez F. Clinical usefulness of biochemical markers of bone turnover in early postmenopausal women: two years longitudinal study [in Spanish]. ***Med Clin (Barc).*** 2008;131:333–338.

105. Garnero P. Markers of bone turnover for the prediction of fracture risk. ***Osteoporos Int.*** 2000;11(suppl 6):S55–S65.

106. Qin YJ, Zhang ZL, Huang QR, He JW, Hu YQ, Li M, Liu YJ. Do the premenopausal daughters of women with postmenopausal osteoporosis have lower peak bone mass?. ***Zhonghua Yi Xue Yi Chuan Xue Za Zhi.*** 2006; 86(6):366-70.

107. Lutz J ,Tesar R. Mother-daughter pairs: spinal and femoral bone densities and dietary intakes. ***Am J Clin Nutr.*** l990;52:872-7.

108. Danielson ME, Cauley JA, Baker CE, Newman AB, Dorman JS, Towers JD, KullerLH.Familial resemblance of bonemineraldensity (BMD) and calcaneal ultrasound attenuation: the BMD in mothers and daughters study. ***J Bone Miner Res***. 1999; 14(1):102-10.

109. Aggarwal N, Raveendran A, Khandelwal N, Sen RK, Thakur JS, Dhaliwal LK, Singla V, Manoharan SR. Prevalence and related risk factors of osteoporosis in peri- and postmenopausal Indian women. ***J Midlife Health***. 2011;2(2):81-5.

110. Khosla S, Riggs BL. Pathophysiology of age-related bone loss and osteoporosis. ***Endocrinol Metab Clin North Am***. 2005; 34(4):1015-30.

111. Marwaha RK, Tandon N, Gupta Y, Bhadra K, Narang A, Mani K, Mithal A, Kukreja S. The prevalence of and risk factors for radiographic vertebral fractures in older Indian women and men: Delhi Vertebral Osteoporosis Study (DeVOS). ***Arch Osteoporos.*** 2012;7(1-2):201-7.

112. Clarke BL, Khosla S.Female reproductive system and bone. ***Arch Biochem Biophys.*** 2010;503(1):118-28.

113. Rosen CJ, Bouxsein ML. Mechanisms of disease: Is osteoporosis the obesity of bone? ***Nat Clin Pract Rheumatol*** 2006;2:35-43.

114. Sharma S, Tandon VR, Mahajan S, Mahajan V, Mahajan A. Obesity: Friend or foe for osteoporosis. ***J Mid-life Health***. 2014;5:6-9.

115. Borer K T. Physical activity in the prevention and amelioration of osteoporosis in women: interaction of mechanical, hormonal and dietary factors. ***Sports Med.*** 2005; 35(9):779-830.

116. Shatrugna V[1], Kulkarni B, Kumar PA, Rani KU, Balakrishna N.Bone status of Indian women from a low-income group and its relationship to the nutritional status. ***Osteoporos Int.*** 2005;16(12):1827-35.

Annexure-I

Individual Information and Consent Form

1. You are being asked to join this research study.

"Bone mineral density and Bone Turnover Markers in healthy pre and postmenopausal women and the influence of multiple risk factors on them"

2. The study is being done by

Principal Investigator (Researcher Study Doctor):

Dr. SahanaShetty, M.D

Department of Endocrine Diabetes and Metabolism,

Christian Medical College

Vellore.

3. DO I HAVE TO TAKE PART IN THIS RESEARCH STUDY?

Your participation is voluntary. This means that you decide whether or not you want to join the study after speaking with the researcher, or other member of the research team.

If you decide to take part you will be asked to sign this consent form. Your signature means that you agree to be a subject in this research.

After reading this form and having a discussion about what it says, you should ask all the questions you want to ask. You should take as much time as you need to make a decision. If you do not understand some of the terms used in this form, ask the person who is discussing the study with you to give any additional information that may make this easier to understand.

Do not sign the form unless you have had all your questions answered and understand exactly what is involved.

The purpose of this research is to find Bone mineral density and Bone Turnover Markers in healthy pre and postmenopausal women and the influence of multiple risk factors on them

4. WHY HAVE I BEEN ASKED TO TAKE PART IN THIS RESEARCH STUDY?

You are being asked to participate as a subject

If you agree to take part in this study you will have tests and examinations to be sure that you qualify for the study.

5. WHY IS THIS RESEARCH STUDY BEING DONE?

This research is being done to Bone mineral density and Bone Turnover Markers in healthy pre and postmenopausal women and the influence of multiple risk factors on them.

6. HOW MANY PEOPLE WILL TAKE PART IN THE RESEARCH STUDY?

You will be one of approximately 80 volunteers who will be participating in this study.

The study will be conducted at Christian Medical College, Vellore.

7. WHAT WILL HAPPEN IF I TAKE PART IN THIS RESEARCH STUDY?

You will undergo medical testing to determine your eligibility at the Clinical Research

Center (CRC), located at Department of Endocrinology Diabetes and Metabolism, Christian Medical college, Vellore-632004.

This visit will include a full history and physical examination, some laboratory blood tests for which a single fasting blood sample will be collected and assessment of bone mineral density by DXA.

8. WHAT ELSE DO I HAVE TO DO?

You must tell the research study doctor about any past and present diseases or medications you are currently taking, including over-the-counter. Remedies and nutritional supplements or herbs.

9. WHAT ARE THE POSSIBLE SIDE EFFECTS, DISCOMFORTS, RISKS OR INCONVENIENCES I CAN EXPECT FROM BEING IN THIS RESEARCH STUDY?

There are no risks in this study,except for minimal radiation exposure with DXA and pain at venepuncture site for collecting blood sample.

10. ARE THERE LIKELY TO BE ANY BENEFITS TO TAKING PART IN THISRESEARCH STUDY?

This study will screen your “Bone mineral density and Bone Turnover Markers in healthy pre and postmenopausal women and the influence of multiple risk factors on them”.

11. WHAT OTHER CHOICES DO I HAVE IF I DO NOT TAKE PART IN THISRESEARCH STUDY?

You may choose not to participate in this study.

12.WHO MAY SEE MY RECORDS?

The research records will be kept private and your name will not be used in any written or verbal reports.

Your research records and medical records may be inspected by members of the research team.

The researcher and research staff will review your medical records and will keep the

Information private.

The research records will be kept in a secured manner and computer records will bepassword protected in Clinical Research Center(CRC) located at the Department of Endocrinology Diabetes andMetabolism,Christian Medical College,Vellore.

The Clinical Research Center staff, as well as the research personnel authorized by the researcher will have access to these records.

The people who reviewed this research study as members of the Christian Medical College Institutional Review Board (IRB) may also review your research and medical records.

The Office of Human Research Protections (OHRP) may also review your research study records.

All of these groups have been requested to keep your name private.

13. WHO CAN ANSWER MY QUESTIONS ABOUT THE STUDY?

If any questions arise related to this research project, or you believe you have any injury related to this study, you can call the researcher above.

You may also call Principal Investigator (Researcher Study Doctor):

Dr. SahanaShetty, M.D, Senior Registrar,

Department of Endocrinology, Diabetes & Metabolism

Christian Medical College, Vellore.

(Telephone: 9944745014, email: sahanashetty0606@gmail.com

14. USE OF IDENTIFIED SPECIMENS FOR FUTURE RESEARCH:

Annexure-I

In addition to the research you are consenting to under this research study, Dr. Sahanashetty ,M.D or other researchers at this or other institutions may wish to study the samples in future research

Information about you may be shared with other researchers who will keep the information confidential. However, it is possible that information about you may become known to people other than the researchers.

15. PARTICIPANT:

PLEASE INDICATE YOUR CHOICE BY INITIALING ONE (1) OF THE FOLLOWINGOPTIONS

___ I consent to have my specimens used for future research studies.

___ I consent to have my specimens used for future research studies only for the study of

_____.

___ I do NOT consent to have my specimens used for future research studies. The specimens will destroyed at the end of the study.

16. PARTICIPANT:

FOR FUTURE CONTACT, PLEASE INITIAL YOUR CHOICES BELOW

I consent to be contacted in the future to learn about:

_____ New research protocols that I may wish to join.

_____ General information about research findings.

_____ Information about the test on my sample that may benefit me or my family members in relation

to choices regarding preventive or clinical care.

_____ I DO NOT AGREE TO BE CONTACTED IN THE FUTURE, EVEN IF THE RESULTS MAY BE IMPORTANT TO MY HEALTH OR MY FAMILY'S HEALTH.

Your wish does not constitute a guarantee that you will be contacted.

17. WHAT ARE MY RIGHTS IF I TAKE PART IN THIS RESEARCH STUDY?

Your participation in this study is voluntary.

You do not waive any of your legal rights by participating in this research study.

Your treatment by doctors and staff at the institution(s) involved in this study, now and in the future, will not be affected in any way if you refuse to participate or if you enter the study and withdraw later.

Annexure-I

INFORMED CONSENT SIGNATURE PAGE

The following is a list of items we discussed about this research study. If you have any questions about any of these items, please ask the person who is discussing the study with You for more information before agreeing to participate.'

What the study is about.

What I must do when I am in the study.

The possible risks and benefits to me.

Who to contact if I have questions or if there is a research related injury.

Any costs and payments.

I can discontinue participating in the study at any time without penalty.

Other choices.

All written and published information will be reported as group data with no reference to my name.

I have been given the name of the researcher and others to contact.

I have the right to ask any questions.

Name of Participant **Signature of Participant**

_____ _____

Address of the participant Date

_____

Name of Person conducting the conducting the **Signature of Person**

Informed Consent Process **Informed Consent Process**

_____ _____

Date

_____

ஒப்புதல் படிவம்

அகசுரப்பியல் பிரிவு கிருத்துவ மருத்துவ கல்லூரி, வேலூர்- 4

1. நீங்கள் இந்த ஆய்வில் பங்கேற்பதற்கு கேட்டு கொள்ளப்பட்டுள்ளீர்.

   இந்த ஆய்வின் தலைப்பு

   "இது எலும்பு குறிப்பான் கொள்முதல் பற்றி சாதாரண குறிப்பு வரம்பு நிறுவுவதற்காக (வகை 1 கொலாஜன்-சி முனையத்தில் டெலேபெப்டைட் - பீட்டா குறுக்கு மடியில்) முன் மாதவிடாய் மற்றும் மாதவிடாய் நின்ற இந்திய பெண்களிடையே - ஒரு குறுக்கு பிரிவு ஆய்வு ஆகும்".

2. இந்த ஆய்வு செய்யும் நபர்:

   முதன்மை ஆய்வாளர் (ஆய்வு மருத்துவர்)

   டாக்டர். சஹானா
   அகசுரப்பியல் நிரிழிவு மற்றும் வளர்சிதைமாற்றம் பிரிவு
   கிருத்துவ மருத்துவ கல்லூரி, வேலூர்-4

3. நான் இந்த ஆய்வில் பங்கேற்க வேண்டுமா ?

தங்களுடைய பங்கேற்பு சுயமானது தாங்கள் ஆய்வாளரிடமோ அல்லது ஆய்வுக் குழுவில் உள்ள வேறு நபர்களிடமோ பேசிய பிறகு இந்த ஆய்வில் பங்கேற்க வேண்டுமா இல்லையா என்பதை முடிவு செய்யலாம்.

தங்களுக்கு பங்கேற்க விருப்பமானால் இந்த படிவத்தில் கையெழுத்திடும்படி கேட்டுக்கொள்ளப்படுவீர்கள். கையெழுத்து தாங்கள் இந்த ஆய்வில் இருக்க சம்மதம் என்பதை வலியுறுத்தம்.

இந்த படிவத்தை படித்து விட்டு இதனில் என்ன கூறப்பட்டுள்ளது என்பதைப் பற்றி விவாதித்த பின்னர் தங்களது சந்தேகங்களைக் கேட்க வேண்டும். தாங்கள் ஒரு முடிவுக்கு வர தங்களுக்கு தேவையான நேரத்தை எடுத்துக் கொள்ளலாம். இந்தப் படிவத்தில் உபயோகிக்கப் படுத்தப்பட்டுள்ள மருத்துவக் குறிப்புகள் ஏதேனும் புரியவில்லை எனில் இப்படிவத்தை உங்களுக்கு விளக்குபவரிடம் இதை எளிதாகப் தெரிந்துக்கொள்ள தேவையான தகவலைக் கேட்டுப் பெறவும்.

தாங்கள் இந்த ஆய்வில் பங்கேற்க உடனே சம்மதம் தெரிவிக்க வேண்டும் என்பதில்லை. பங்கேற்க வேண்டுமா வேண்டாமா என்பதை முடிவு செய்ய தேவையான அளவு நேரம் எடுத்துக்கொள்ளவும்.

இந்த படிவத்தின் ஒரு பிரதியை எடுத்துக் சென்று தங்களுடைய குடும்பத்தினரிடமும் நண்பர்களிடமும் கலந்து ஆலோசித்து பின்னர் முடிவேடுக்கலாம்.

தாங்கள் பங்கேற்க வேண்டாம் என்று முடிவு செய்தாலும் தங்களுடைய நலன் கருதி முறையான சிகிச்சை அளிப்பார்கள். தாங்கள் பங்கேற்க இசைந்தாலும் இல்லையேன்றாலும் இப்படிவத்தின் ஒரு பிரதி உங்களுக்கு அளிக்கப்படும்.

தாங்கள் அனைத்து கேள்விகளுக்கும் பதில் கிடைக்காமலோ? ஆய்வில் என்ன நடக்கப்போகிறது என்பதை தெளிவாக அறியாமலோ இப்படிவத்தில் கையேழுத்திட வேண்டாம். தாங்கள் இந்த ஆய்வில் பங்கேற்க முடிவு செய்தாலும் எந்த நேரத்திலும் காரணம் கூறாமல் இந்த ஆய்வில் இருந்து விலகிக் கொள்ளலாம். இதனால் தங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்த வித பாதிப்பும் இருக்காது. மேலும் தொடர்ந்து இங்கேயே சிகிச்சைப் பெற்றுக் கொள்ளலாம்.

4.நான் ஏன் இந்த ஆய்வில் கலந்து கொள்ள கேட்டுக் கொள்ளப் பட்டிருக்கிறேன்?
இந்த ஆய்வில் தாங்கள் கலந்துக்கொள்ள சம்மதம் தெரிவித்தால் நீங்கள் இந்த ஆய்வுக்கு தகுதி உடையவரா என்பதை உறுதி செய்யும் பொருட்டு சில பரிசோதனைகள் செய்யப்படும்.

5.இந்த ஆய்வு ஏன் நடத்தப்படுகிறது-
இந்த ஆய்வின் நோக்கம் “இது எலும்பு குறிப்பான் கொள்முதல் பற்றி சாதாரண குறிப்பு வரம்பு நிறுவுவதற்காக (வகை 1 கொலாஜன்-சி முனையத்தில் டெலேபெப்டைட் - பீட்டா குறுக்கு மடியில்) முன் மாதவிடாய் மற்றும் மாதவிடாய் நின்ற இந்திய பெண்களிடையே - ஒரு குறுக்கு பிரிவு ஆய்வு ஆகும்”.

6.எத்தனை பேர் இந்த ஆய்வில் பங்கேற்க உள்ளனர்?
ஏறக்குறைய உங்களுடன் சேர்த்து இந்த ஆய்வில் 80 பேர் பங்கேற்பார்கள். இந்த ஆய்வு வேலூர் கிருத்துவ மருத்துவக் கல்லூரியில் நடைபெறும்.

7.நான் இந்த ஆய்வில் பங்கேற்பதானால் எனக்கு என்ன நடக்கும்?
வேலூர் கிருத்துவ மருத்துவக் கல்லூரியின் அகசுரப்பியல் நிரிழிவு மற்றும் வளர்சிதைமாற்றம் பிரிவில் அமைந்துள்ள ஆய்வு அறையில் கீழே கொடுக்கப்பட்டுள்ள பரிசோதனைகள் செய்ப்பட்டு ஆய்வுக்கான தகுதி அறியப்படும்.

இந்த வருகையின் போது தங்களுடைய மருத்துவ வரலாறு அறியப்பட்டு, உடற்பரிசோதனை, இரத்த அழுத்தம், டெக்ஸா ஸ்கேன் - இது எலும்பின் அடர்த்தியை பற்றி அறிய எடுக்கப்படும் ஸ்கேன். இந்த ஸ்கேன் குறைந்த கதிர்வீச்சையுடையது மற்றும் சில ஆய்வுகூடப் பரிசோதனைகளும் செய்யப்படும்.

இரத்த மாதிரி எடுப்பதற்காக ஆய்கூடத்திற்கு நீங்கள் காலி வயிற்றுடன் (முந்தைய நாள் இரவு 11 மணிக்கு பிறகு எதுவும் உண்ணாமல்) வரவேண்டும்.

8.நான் வேறு என்ன செய்ய வேண்டும்?

நீங்கள் ஆய்வு மருத்துவரிடம் உங்களுக்கு இப்பொழுது இருக்கின்ற மற்றும் முன்னர் இருந்த நோய்களைப் பற்றியும் ஒவ்வாமையைப் பற்றியும் கூற வேண்டும். நீங்கள் உட்கொள்ளும் மருந்துகள் பற்றியும் கூற வேண்டும்.

9.இந்த ஆய்வில் பங்கேற்பதனால் எனக்கு என்னேன்ன பக்கவிளைவுகள் ஏற்பட வாய்ப்பு இருக்கிறது?

இந்த ஆய்வில் பங்கேற்பதால் உங்களுக்கு பக்கவிளைவுகள் இல்லை. டெக்ஸா ஸ்கேன் குறைந்த கதிர்வீச்சையுடையது மற்றும் இரத்த மாதிரி எடுப்பதால் தமணியில் சிறு வலி ஏற்பட வாய்ப்புள்ளது.

10.இந்த ஆய்வில் பங்கேற்பதால் எனக்கு ஏதாவது நன்மைகள் உண்டா?

இந்த ஆய்வு உங்களுக்கு நேரடி ஆதாயம் தரும். டெக்ஸா ஸ்கேன் மூலமாக நீங்கள் எலும்பின் அடர்த்தியை பற்றி தெரிந்து கொள்ள வாய்ப்புள்ளது. இந்த ஆய்வானது எலும்பு குறிப்பான் கொள்முதல் பற்றி சாதாரண குறிப்பு வரம்பு நிறுவுவதற்காக (வகை 1 கொலாஜன்-சி முனையத்தில் டெலேபெப்டைட் - பீட்டா குறுக்கு மடியில்) முன் மாதவிடாய் மற்றும் மாதவிடாய் நின்ற இந்திய பெண்களிடையே - ஒரு குறுக்கு பிரிவு ஆய்வு ஆகும்.

11.நான் இந்த ஆய்வில் பங்கேற்கவில்லை என்றால் எனக்கு வேறு என்ன வழிகள் உள்ளது?

நீங்கள் இந்த ஆய்வில் பங்கேற்காமல் இருக்கலாம்.

12.எனது ஆய்வு தகவல்களை யார் பார்ப்பார்கள்?

உங்கள் ஆய்வு அறிக்கை ரகசியமாக பாதுகாக்கப்படும் உங்கள் பெயர் வெளியிடப்படமாட்டாது.

உங்கள் ஆய்வு அறிக்கை மற்றும் மருத்துவ அறிக்கை ஆய்வு குழுவால் சோதிக்கப்படும்.

ஆய்வு குழு உங்கள் ஆய்வு அறிக்கையை சோதித்து விட்டு தகவல்கள் ரகசியமாக பாதுகாக்கப்படும்.

ஆய்வு அறிக்கை பத்திரமாக பாதுகாக்கப்படும் கணினி அறிக்கைகள் ரகசிய குறியேட்டு எண்ணாள் பாதுக்கப்படும்.

ஐ.ஆர்.பி. நபர்கள் உங்கள் ஆய்வு அறிக்கையை சோதிப்பார்கள் ஒ.பச்.ஆர்.பி. நபர்கள் உங்கள் ஆய்வு அறிக்கையை சோதிப்பார்கள் குழவின் நபர்கள் உங்கள் தகவலை ரகசியமாக பாதுகாப்பார்கள்.

13.ஆய்வைப் பற்றிய என்னுடைய கேள்விகளுக்கு யார் பதிலளிப்பார்கள்?

இந்த ஆய்வைப் பற்றி உங்களுக்கு ஏதேனும் கேள்விகள் தோன்றினாலோ அல்லது இந்த ஆய்வினால் உங்களுக்கு ஏதேனும் பாதிப்பு ஏற்பட்டிருக்கிறது என்று நீங்கள் கருதினாலோ மேலே கூறப்பட்டுள்ள ஆய்வாளரை நீங்கள் உடனே தொடாபு கொள்ளலாம்.

ஆய்வின் மைய ஆய்வாளவரையும் (ஆய்வு மருத்துவர்) நீங்கள் தொடர்பு கொள்ளலாம்.

டாக்டர். சஹானா
அகசுரப்பியல் நிரிழிவு மற்றும் வளர்சிதைமாற்றம் பிரிவு
கிருத்துவ மருத்துவ கல்லூரி, வேலூர்-4
தொலைப்பேசி எண்: 9944745014

14.தங்களுடைய மாதிரிகளை எதிர்கால ஆராய்ச்சிகளுக்கு பயன்படுத்துவார்களா?
தங்களைப் பற்றிய தகவல்களை இரகசியமாக வைப்பார்கள். ஆயினும் சில சமயங்களில் தங்களைப் பற்றிய தகவல் ஆய்வாளர்களைத் தவிர மற்றவர்களுக்கும் தெரிய வரலாம். இந்த ஆய்வுக் குறிப்புகள் டாக்டர் சஹானா தவிர இங்கு உள்ள மருத்துவ ஆராய்ச்சியாளர்கள் எதிர்காலத்தில் பயன் படுத்துவார்கள்.

15.பங்கேற்பாளர்:

தங்களுடைய விருப்பத்தை கீழ்க்கண்ட ஏதேனும் ஒரு வாக்கியத்தில் கையேழத்திட்டு தெரிவிக்கவும்.

- என்னுடைய மாதிரிகளை எதிர்கால ஆய்வுக்கு பயன்படுத்திக்கொள்ள சம்மதிக்கிறேன்.
- என்னுடைய மாதிரிகளை எதிர்காலத்தில் இந்த - எலும்பு குறிப்பான் கொள்முதல் பற்றி சாதாரண குறிப்பு வரம்பு நிறுவுவதற்காக (வகை 1 கொலாஜன்-சி முனையத்தில் டெலேபெப்டைட் - பீட்டா குறுக்கு மடியில்) முன் மாதவிடாய் மற்றும் மாதவிடாய் நின்ற இந்திய பெண்களிடையே - ஒரு குறுக்கு பிரிவு ஆய்வுக்கு மட்டும் பயன்படுத்திக்கொள்ள சம்மதிக்கிறேன்
- என்னுடைய மாதிரிகளை எதிர்கால ஆய்பவுக்கு பயன்படுத்த சம்மதம் இல்லை.

16.பங்கேற்பாளர்:

எதிர்காலத்தில் தொடர்பு கொள்ள, கீழே கொடுக்கப்பட்டுள்ள வாய்ப்புகளில் விருப்பமானவற்றை கையேழத்திட்டு எதிர்காலத்தில் கீடிக்கண்டவற்றைப் பற்றி அறிந்து கொள்ள என்னைத் தொடர்பு கொள்ள சம்மதிக்கிறேன்.

- நான் பங்கேற்கக் கூடிய புதிய ஆய்வு வரைமுறைகளைப் பற்றி அறிய
- ஆய்வு முடிகளைப் பற்றி பொதுவான தகவல்களை அறிந்து கொள்ள
- எனக்குகோ எனது குடும்பத்தினருக்கோ மருத்து பயனளிக்குமா என்பதை அறிய

- என்னுடைய உடல் நலத்திற்கோ என்னுடைய குடும்பத்தினர் உடல் நலத்திற்கோ பயனளிக்கக்கூடியதாக இருந்தாலும் எதிர்காலத்தில் என்னைத் தொடர்பு கொள்வதில் எனக்கு சம்மதமில்லை.

உங்களுடைய விருப்பம் கண்டிப்பாக எதிர்காலத்தில் நீங்கள் தொடர்பு கொள்ளப்படுவீர்கள் என்பதற்கு உத்திரவாதம் இல்லை.

17.இந்த ஆய்வில் பங்கேற்பதால் எனக்கு கிடைக்கும் உரிமைகள் என்ன?

நீங்கள் இந்த ஆய்வில் பங்கேற்பது உங்கள் சுய விருப்பபும் இந்த ஆய்வில் பங்கேற்பதால் உங்களுடைய சட்ட புர்வமான எந்த ஒரு உரிமையையும் நீங்கள் இழக்கபபோவது இல்லை.

நீங்கள் இந்த ஆய்வில் பங்கேற்க சம்மதித்து விட்டு பிறகு விளகிக் கொண்டாலும் உங்களுக்கு இந்த மருத்துவமனையில் முறையான சிகிச்சை கிடைக்கும்

தகவலறிந்த ஒப்புதல் கையொப்பம் பக்கம்

இந்த ஆய்வை பற்றி உங்களுக்கு விளக்கப்பட்ட அனைத்து தலைப்புகளும் கீழே கொடுக்கப்பட்டுள்ளது. உங்களுக்கு ஏதேனும் ஒரு தலைப்பில் சந்தேகம் இருந்தால் இந்த ஆய்வை பற்றி உங்களிடம் விளக்கும் நபரிடம் அதைப் பற்றி தெளிவாக கேட்டு தெரிந்து கொள்ளவும்.

இந்த ஆய்வு எதைப்பற்றியது ?

இந்த ஆய்வில் இருக்கும்பொழுது நான் என்ன செய்ய வேண்டும்.

இந்த ஆய்வில் பங்கேற்பதால் எனக்கு கிடைக்கப் போகும் நன்மைகள் மற்றும் தீமைகள் எனக்கு இந்த ஆய்வைப்பற்றி எந்த சந்தேகமோ அல்லது உடல்நல குறைபாடோ இருந்தால் நான் எந்த நபரை தொடர்பு கொள்ள வேண்டும்.

நான் இந்த ஆய்வில் இருந்து எந்த வித நிபந்தனைளும் இன்றி எப்போது வேண்டுமானாலும் விளகிக் கொள்ளலாம் இந்த ஆய்வைப் பற்றி தகவல்கள் அனைத்தும் ஒரு கூட்டாகவே வெளியிடப்படும் என்னைப் பற்றிய சுய அடையாலம் ரகசியமாக பாதுகாக்கப்படும்.

ஆய்வு மருந்தை எப்படி எடுக்க வேண்டும் என்ற நேர அட்டவனை இருந்தால் அந்த நேர அட்டவணை எனக்கு கொடுக்கப்படும்.

ஆய்வுக் குழுவைப்பற்றிய தகவல்கள் எனக்கு கொடுக்கப்பட்டுள்ளது.

ஆய்வைப் பற்றிய கேள்விகளை கேட்க எனக்கு முழு உரிமை உள்ளது.

பங்கேற்பாளரின் பெயர் பங்கேற்பாளரின் கையொப்பம்

....

பங்கேற்பாளரின் கைநாட்டு: ..... தேதி: .....

பங்கேற்பாளரின் விலாசம்:.....

ஒப்புதல் படிவத்தை பங்கேற்பாளருக்கு விளக்கும் நபரின் பெயர்

ஒப்புதல் படிவத்தை பங்கேற்பாளருக்கு விளக்கும் நபரின் கையேப்பம்

....

Annexure-III

DIETARY RECALL

MEALPLAN

MealTime	FoodGroup	Raw	Cooked Recipe	Servings Amounts
Breakfast	Milk	100ml	Milkor	1 Cup
	Sugar	15g	Teaor	1 Cups
			Coffee	1 Cup
	Cereals	70g	BreakfastItem	
	Pulses	20g		
Lunch	Cereals	120g	Rice	2 Cups
	Greens	50 g	Pulkas	2 Nos.
	Pulses	20 g	Dhal	1/2 Cup
	Vegetables	150g	Veg.curry	3/4 Cup
	Vegetables	50g	Veg.salad	7-8 Slices
	Milk	100ml	Curd	1/2 Cup
Tea	Cereals	50g	Snack	
	Milk	50ml	Tea	1 Cup
	Sugar	10 g		
Dinner	Cereals	120g	Rice	2 Cups
	Greens	50 g	Pulkas	2 Nos.
	Pulses	20g	Dhal	1/2 Cup
	Vegetables	150g	Veg.curry	3/4 Cup
	Milk(Curd)	50ml		
	Vegetables	50g		
	Fruit	100g	Seasonal	1 Medium

Note:ForNon-Vegetarians: egg- daily / weekly (once or twice) / monthly (once /twice)
Meat - daily / weekly (once or twice) / monthly (once /twice)
Chicken daily / weekly (once or twice) / monthly (once /twice)
Fish- daily / weekly (once or twice) / monthly (once /twice)

BreakfastItems: Idli-4Nos./Dosa-3Nos./Upma-1-1/2Cup

Snacks: Toast -2Slices /Samosa-2/Sandwiches-2 /Biscuits-5.

Fruits :

Members

Adults

Children

Cooking oil per month -

Annexure-IV

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

Annexure-IV

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ hours per day
_____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ days per week

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ hours per day
_____ minutes per day

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ days per week

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ hours per day
_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ days per week

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ hours per day
_____ minutes per day

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ hours per day
_____ minutes per day

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ days per week

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ hours per day
_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ days per week

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ days per week

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ hours per day
_____ minutes per day

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ days per week

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ hours per day
_____ minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ hours per day
_____ minutes per day

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ hours per day
_____ minutes per day

This is the end of the questionnaire, thank you for participating.

Physical activity (IPAQ)		
Work		
Transport		
Domestic		
Leisure		
Grading -	**Total**	

Annexure-V

PROFORMA

Name: Hospital. No. :

Age: Sex:

Address: Phone no:

Date of enrollment:

Socio-economic strata:

Score	**Education**	
7	Profession or honours	
6	Graduate of Post graduate	
5	Intermediate or post high school diploma	
4	High school certificate	
3	Middle school certificate	
2	Primary school certificate	
1	Illiterate	
Score	**Occupation**	
10	Profession	
6	Semi-profession	
5	Clerical, Shop-owner, farmer	
4	Skilled worker	
3	Semi-skilled worker	
2	Unskilled worker	
1	Unemployed	
Score	**Family income in Rs.**	
12	>30,375/-	
10	15,188/- to 30, 374/-	
6	11,362/- to 15,187/-	
4	7,594/- to 11,361/-	
3	4,556/- to 7,593/-	
2	1,521/- to 4,555/-	
1	< 1,520/-	
Total	**Socioeconomic class**	
26-29	Upper -1	
16-25 middle	Upper middle – 2	
11-15	Lower middle – 3	
5-10 lower	Upper lower – 4	
< 5	Lower - 5	
	Total	

Annexure-V

HISTORY

Menstrual cycles	
Last Menstrual Period:	
Age of menopause (in postmenoupausal subjects)	
Hormone replacement therapy	
History of fractures	
Dietary calcium intake	separate sheet - enclosed
Duration of sunlight exposure & areas of skin exposed	
History of Smoking	
History of Alcohol	
History of tobacco consumption	
History of steroid use (prednisolone >5 mg daily for 3 months current or past)	
Rheumatoid arthritis (physician confirmed)	
Secondary osteoporosis	
Treatment for osteoporosis	
Drug history : Anti TB /Anti convulsant / Steroids	
Family history of fracture	
Comorbidities	
Birth weight	
GENERAL PHYSICAL EXAMINATION- ANTHROPOMETRY	
Height	
Weight	
Body mass Index	
Waist circumference	
Blood Pressure	
Pulse	
Proximal muscle weakness	
Any significant findings (Pallor, IcterusEtc)	

Annexure-V

INVESTIGATIONS:

Biochemistry

Albumin corrected calcium

Phosphorus

Alkaline phosphatase

Creatinine

Intact Paratharmone (iPTH)

25(OH) Vitamin-D

Bone mineral density

Spine(L1-L4) , femur neck, distal forearm

Bone turnover markers (BTMs)

Bone resorption markers:

Serum C-Terminal Telopeptides Type I Collagen **(CTX)**

Urine DeoxyPyridinoline**(DPD*)***

Bone formation markers:

Serum Osteocalcin **(OCN)**

Serum Procollagen Type 1 N-terminal propeptide **(P1NP)**

sno	id	name	cmch	relation	age	meno_age	ht	wt	BMI	PARITY	Diet_Cal	Phy_activity	SES	sun light	creat	calcium	phosph	ALP	albumin	S.CTX	S.P1NP	S.OC	Ur.DPD	Vit.D	PTH	LS_BMD	LS_T	LS_Z	LT_FN_BMD	LT_FN_T	LT_FN_Z	FA_BMD	FA_Ts	FA_Zc
1	a	Sundarammal	677042F	mother	60	40	154	79	33.31	6	480	864	4	60	0.86	8.80	3.40	101.00	4.00	306.60	34.50	19.90	9.92	22.50	49.30	0.948	-0.9	0.6	0.748	-0.9	0.4	0.572	-0.1	1.1
1	b	Hemavathy	677044F	daughter	38		162	68	25.91	3	530	7590	4	20	0.75	8.90	3.70	67.00	4.50	225.00	31.90	13.40	9.53	22.00	30.20	1.002	-0.4	-0.3	0.842	-0.1	0.2	0.641	1.1	1.4
2	a	Datchayani	676763F	mother	64	45	157	55	22.31	2	590	977	5	15	1.01	9.10	3.80	95.00	4.30	642.30	44.70	22.40	14.20	25.34	29.40	0.665	-3.5	-1.7	0.646	-1.8	-0.3	0.403	-3.3	-1.7
2	b	Kavitha	676760F	daughter	36		157	55	22.31	5	670	6609	4	60	0.95	8.40	3.30	58.00	4.10	445.20	61.10	20.60	7.71	13.72	52.30	1.100	0.5	0.6	0.905	0.5	0.7	0.596	0.3	0.5
3	a	Ellammal	678209F	mother	65	35	158	50	20.03	6	680	2940	5	15	1.02	8.50	4.10	95.00	4.00	856.60	78.60	38.30	15.02	28.22	39.50	0.749	-2.7	-0.9	0.650	-1.8	-0.2	0.403	-3.3	-1.6
3	b	Mangammal	634638F	daughter	32		158	70	28.04	2	500	5421	4	10	0.88	8.60	3.30	71.00	4.00	368.60	63.00	28.50	8.01	18.22	52.60	0.958	-0.8	-0.8	0.672	-1.6	-1.5	0.550	-0.5	-0.4
4	a	Vanaja	678172F	mother	58	40	135	57	31.28	5	580	3075	5	60	0.79	8.60	3.20	96.00	4.30	649.80	74.50	29.50	10.36	17.01	59.80	0.748	-2.7	-1.4	0.604	-2.2	-1.0	0.437	-2.6	-1.5
4	b	Valarmathi	678154F	daughter	38		146	55	25.80	3	740	11340	4	30	0.74	8.40	3.40	73.00	4.20	326.00	39.90	16.30	8.60	23.70	44.30	0.951	-0.9	0.7	0.756	-0.8	-0.6	0.535	-0.8	-0.5
5	a	Manjula	678167F	mother	56	46	150	65	28.89	5	440	5142	5	30	0.74	8.30	3.60	69.00	4.20	348.00	43.80	18.50	8.40	18.50	93.00	0.906	-1.3	-0.1	0.678	-1.5	-0.4	0.532	-0.9	0.1
5	b	Kowsalaya	678160F	daughter	32		162	64	24.39	1	450	5100	4	30	0.83	8.60	3.30	51.00	4.20	380.90	53.30	21.50	8.26	18.80	50.20	0.981	-0.6	-0.6	0.883	0.3	0.4	0.512	-1.2	-1.1
6	a	Savithri	678182F	mother	60	50	158	66	26.44	3	570	7377	5	30	1.11	8.60	4.20	81.00	4.00	918.40	67.60	32.90	10.00	23.90	112.50	0.926	-1.1	0.4	0.592	-2.3	-1.0	0.517	-1.1	0.1
6	b	Sumathi	678197F	daughter	34		150	77	34.22	5	600	5440	4	45	0.79	8.80	3.60	99.00	4.20	389.70	54.80	22.30	12.90	14.90	102.60	1.109	0.6	0.6	0.743	-1.0	-0.8	0.533	-0.9	-0.7
7	a	Vijaya	681996F	mother	51	48	138	49	25.73	3	420	5282	4	60	0.83	9.70	3.50	87.00	4.20	236.50	27.70	16.40	8.81	22.93	21.80	0.827	-2.0	-1.2	0.842	-0.1	0.8	0.531	-0.9	-0.1
7	b	Tamilarasi	681998F	daughter	33		150	57	25.33	3	590	4634	4	60	0.77	10.00	4.60	83.00	5.00	513.80	93.90	49.80	12.10	15.00	22.30	0.803	-2.2	-2.2	0.703	-1.3	-1.2	0.584	0.1	0.3
8	a	Vasantha	681392F	mother	55	50	148	55	25.11	4	470	1818	4	120	0.90	9.10	4.20	111.00	4.10	563.90	67.80	42.80	9.30	15.60	31.20	0.781	-2.4	-1.3	0.558	-2.6	-1.5	0.458	-2.2	-1.3
8	b	Elamathi	681394F	daughter	34		151	65	28.51	2	540	5996	4	60	0.74	9.30	3.70	56.00	4.30	246.50	27.50	14.70	9.94	14.90	25.10	1.053	0.1	0.1	0.772	-0.7	-0.5	0.705	0.2	0.4
9	a	Mannikammal	681390F	mother	60	50	148	44	20.09	5	510	1260	5	60	0.95	9.20	3.70	77.00	4.00	688.40	65.60	42.30	12.26	18.00	45.30	0.668	-3.4	-2.0	0.550	-2.7	-1.4	0.376	-3.8	-2.5
9	b	Saraswathi	478905F	daughter	40		150	62	27.56	3	510	4578	4	60	0.88	9.60	4.10	65.00	3.80	371.80	40.60	20.50	9.44	28.60	35.50	0.803	-2.2	-2.0	0.849	0.0	0.3	0.597	0.3	0.7
10	a	Lakshmi	682006F	mother	58	40	143	63	30.81	10	590	360	5	20	0.75	9.20	3.80	90.00	9.30	526.70	51.90	22.00	13.78	12.00	102.40	0.759	-2.6	-1.3	0.675	-1.6	-0.3	0.530	-0.9	0.2
10	b	Malar	682001F	daughter	35		140	44	22.45	2	510	7755	4	10	0.75	9.00	4.00	103.00	4.40	329.30	48.30	20.20	7.70	16.70	53.10	0.924	-1.1	-1.0	0.700	-1.3	-1.2	0.536	-0.8	-0.6
11	a	Amutha	683362F	mother	58	55	150	59	26.22	2	500	2295	4	10	0.82	9.80	4.20	75.00	4.70	885.90	88.00	31.00	16.56	17.40	64.50	0.869	-1.6	-0.3	0.692	-1.4	-0.2	0.530	-0.9	0.2
11	b	Shakila	683356F	daughter	33		155	64	26.64	2	500	2250	4	20	0.94	9.20	3.90	52.00	4.20	413.70	32.60	18.90	9.57	14.00	72.50	1.151	0.9	1.0	0.825	-0.2	-0.1	0.536	-0.8	-0.6
12	a	Vijaya	006079C	mother	55	45	145	60	28.54	4	520	11414	4	30	0.88	9.70	3.90	78.00	4.40	729.70	55.30	28.30	15.96	11.30	63.30	0.915	-1.2	-0.1	0.645	-1.8	-0.7	0.579	0.0	1.0
12	b	Sangeetha	683366F	daughter	34		157	64	25.96	3	540	1890	4	20	0.86	9.10	3.40	62.00	4.10	321.00	31.40	15.60	7.30	12.00	55.40	0.967	-0.7	-0.7	0.762	-0.8	-0.6	0.578	0.0	0.2
13	a	Lakshmi	363967B	mother	65	40	150	64	28.44	2	630	4146	5	20	0.67	8.90	3.60	89.00	4.00	474.10	47.70	18.80	13.97	25.60	41.10	0.875	-1.6	0.2	0.665	-1.7	-0.1	0.468	-2.1	-0.4
13	b	Selvi	683376F	daughter	39		153	59	25.20	5	500	8982	3	300	0.88	8.90	3.50	71.00	4.20	265.20	32.40	15.90	13.00	22.90	33.20	0.958	-0.8	-0.6	0.891	0.4	0.6	0.578	0.0	0.3

sno	id	name	cmch	relation	age	meno_age	ht	wt	BMI	PARITY	Diet_Cal	Phy_activity	SES	sun light	creat	calcium	phosph	ALP	albumin	S.CTX	S.P1NP	S.OC	Ur.DPD	Vit.D	PTH	LS_BMD	LS_T	LS_Z	LT_FN_BMD	LT_FN_T	LT_FN_Z	FA_BMD	FA_Ts	FA_Zc
14	a	Mary	683373F	mother	63	40	151	47	20.61	6	650	4740	5	240	1.12	9.70	3.90	87.00	4.20	824.20	37.80	23.20	6.91	24.00	13.00	0.789	-2.3	-0.7	0.661	-1.7	-0.2	0.431	-2.7	-1.3
14	b	Santhi	683368F	daughter	30		160	60	23.44	3	560	7176	4	10	1.16	9.60	3.70	56.00	4.50	549.40	38.50	21.60	7.68	23.00	22.70	0.986	-0.6	-0.5	0.902	0.5	0.6	0.561	-0.3	-0.2
15	a	Radha	686295F	mother	60	45	138	36	18.90	4	540	3564	5	20	0.87	9.10	4.40	101.00	4.50	1,095.00	136.10	51.40	13.65	23.00	62.50	0.611	-4.0	-2.5	0.472	-3.4	-2.1	0.542	-0.7	-0.3
15	b	Nirmala	686298F	daughter	42		145	60	28.54	5	600	3585	2	20	0.89	8.70	2.70	76.00	4.20	215.20	33.50	12.80	7.87	14.60	116.00	0.816	-2.1	-1.8	0.889	0.4	0.7	0.542	-0.7	-0.3
16	a	Navaneetham	686293F	mother	53	40	158	60	24.03	3	620	1306	4	30	1.18	10.30	3.80	81.00	4.50	641.70	47.60	28.30	8.94	29.40	34.40	0.839	-1.9	-0.9	0.699	-1.4	-0.4	0.453	-2.3	-1.5
16	b	Nalini	686292F	daughter	38		155	60	24.97	1	590	9594	4	10	1.06	10.00	3.20	64.00	4.20	324.20	49.80	18.20	7.50	6.10	41.70	1.087	0.4	0.5	0.853	0.0	0.3	0.591	0.2	0.5
17	a	Jeyammal	686291f	mother	63	43	156	58	23.83	3	630	3780	5	60	0.96	9.70	3.70	87.00	4.60	342.70	34.70	12.70	6.90	20.40	40.20	0.904	-1.3	0.4	0.657	-1.7	-0.3	0.514	-1.2	0.3
17	b	Jeyanthi	686285F	daughter	36		155	54	22.48	4	570	4340	4	60	1.02	10.00	2.80	64.00	4.40	209.00	39.40	13.00	7.53	29.20	26.20	0.995	-0.5	-0.4	0.788	-0.5	-0.3	0.547	-0.6	-0.3
18	a	Lakshmiammal	686283F	mother	65	50	154	53	22.35	3	690	1519	5	60	0.92	10.10	4.00	96.00	4.40	960.70	53.50	32.10	14.70	25.10	77.70	0.643	-3.7	-1.9	0.480	-3.3	-1.8	0.283	-5.5	-3.8
18	b	Vendamani	686301F	daughter	45		154	48	20.24	3	650	4866	5	60	0.97	10.40	4.00	73.00	4.70	1,140.00	66.50	31.50	11.30	17.00	49.60	0.925	-1.1	-0.6	0.759	-0.8	-0.3	0.437	-2.6	-2.1
19	a	Gnanammal	689523F	mother	60	45	149	56	25.22	7	640	3478	5	20	0.77	9.00	3.80	96.00	4.20	711.70	46.40	25.60	10.25	15.00	19.90	0.533	-4.7	-3.2	0.659	-1.7	-0.4	0.372	-3.8	-2.6
19	b	Kasthuri	689525F	daughter	40		150	56	24.89	3	640	9640	4	180	0.82	9.00	3.60	111.00	4.10	250.10	27.50	13.50	7.44	27.55	15.40	0.868	-1.6	-1.4	0.742	-1.0	-0.7	0.488	-1.7	-1.3
20	a	Lakshmi	689515F	mother	64	50	152	64	27.70	3	630	5373	5	120	0.71	8.20	3.10	90.00	4.10	918.00	41.10	19.00	10.24	23.00	35.50	0.800	-2.2	-0.5	0.738	-1.0	0.5	0.404	-3.2	-1.7
20	b	Rukmani	689516F	daughter	32		159	54	21.36	5	560	4961	5	20	0.87	8.50	3.30	62.00	4.60	546.60	45.50	22.10	9.05	15.00	45.80	0.976	-0.6	-0.6	0.808	-0.4	-0.2	0.591	0.2	0.4
21	a	Malarkodi	690367F	mother	58	50	155	67	27.89	3	590	3006	4	30	0.72	9.40	3.90	73.00	4.60	246.50	38.60	18.30	14.03	13.70	57.50	0.951	-0.9	0.4	0.761	-0.8	0.4	0.507	-1.3	-0.2
21	b	Kotteswari	690372F	daughter	35		156	60	24.65	3	550	5660	4	180	0.75	9.20	3.40	81.00	4.50	728.10	51.20	22.00	7.80	28.00	58.20	0.771	-2.5	-2.4	0.674	-1.6	-1.4	0.549	-0.6	-0.3
22	a	Vasantha	689521F	mother	62	45	153	54	23.07	3	640	3800	5	300	0.85	9.10	3.90	75.00	4.30	458.80	40.40	25.80	10.53	23.10	34.60	0.739	-2.8	-1.2	0.686	-1.5	-0.1	0.403	-3.3	-1.8
22	b	Kamatchi	689522F	daughter	40		156	68	27.94	2	640	7680	4	300	0.89	9.10	4.00	67.00	4.50	221.30	29.30	10.40	9.53	20.20	34.50	1.007	-0.4	-0.1	0.955	1.0	1.3	0.591	0.2	0.6
23	a	Saroja	689517F	mother	60	50	146	44	20.64	3	550	2880	5	20	1.12	9.30	4.60	139.00	3.90	547.70	88.40	41.50	21.20	22.00	16.70	0.577	-4.3	-2.8	0.455	-3.6	-2.2	0.232	-6.4	-5.2
23	b	Bharathi	689518F	daughter	35		145	67	31.87	3	640	4233	4	20	0.92	9.50	4.20	78.00	4.40	757.00	64.70	25.00	14.02	17.00	23.30	0.907	-1.3	-1.2	0.806	-0.4	-0.2	0.505	-1.4	-1.1
24	a	Shamshud Begu	883802	mother	58	48	154	75	31.62	3	730	945	2	10	1.05	9.20	3.60	59.00	3.90	538.70	27.10	16.70	15.42	23.00	62.20	1.081	0.3	1.6	0.846	0.0	1.2	0.591	0.2	1.4
24	b	Nazia Tabusum	503427B	daughter	30		162	73	27.82	3	670	2520	2	10	0.76	8.40	3.70	34.00	4.00	295.20	31.30	15.10	9.87	7.10	75.10	1.072	0.2	0.2	0.799	-0.5	-0.4	0.604	0.5	0.6
25	a	Lakshmi	691276F	mother	56	40	145	47	22.35	3	510	2250	5	240	0.72	9.00	3.90	62.00	4.30	381.70	33.60	18.30	8.85	27.00	83.60	0.994	-0.5	0.7	0.640	-1.9	-0.8	0.438	-2.6	-1.6
25	b	Bhanumathi	691275F	daughter	42		162	60	22.86	4	650	3195	4	120	0.90	8.80	3.90	58.00	4.20	183.50	24.50	11.00	6.83	16.00	32.30	1.045	0.0	0.3	0.768	-0.7	-0.4	0.550	-0.5	-0.1
26	a	Neelammal	691853F	mother	60	42	145	57	27.11	4	450	1755	4	10	0.87	9.30	3.80	78.00	4.10	900.60	66.90	21.80	11.00	13.10	60.80	0.611	-4.0	-2.5	0.527	-2.9	-1.6	0.436	-2.7	-1.4
26	b	Indirani	691855F	daughter	40		116	67	29.00	3	650	4970	5	10	0.74	9.30	2.60	59.00	4.40	456.00	36.90	17.20	10.55	24.70	51.90	0.936	-1.0	-0.8	0.644	-1.8	-1.5	0.577	0.0	0.3

sno	id	name	cmch	relation	age	meno_age	ht	wt	BMI	PARITY	Diet_Cal	Phy_activity	SES	sun light	creat	calcium	phosph	ALP	albumin	S.CTX	S.P1NP	S.OC	Ur.DPD	Vit.D	PTH	LS_BMD	LS_T	LS_Z	LT_FN_BMD	LT_FN_T	LT_FN_Z	FA_BMD	FA_Ts	FA_Zc
27	a	Saroja	691279F	mother	65	45	159	69	27.29	3	620	1143	5	60	0.87	9.80	3.90	91.00	4.30	435.20	51.10	26.20	9.34	17.00	37.20	0.736	-2.8	-1.0	0.622	-2.0	-0.5	0.422	-2.9	-1.3
27	b	Vijaya	691277F	daughter	46		160	89	34.77	2	550	1620	4	120	0.79	9.30	3.90	95.00	3.70	688.20	95.00	28.60	13.02	26.00	24.10	0.958	-0.8	-0.3	0.765	-0.8	-0.2	0.592	0.2	0.8
28	a	Ananthi	691272F	mother	55	40	150	78	34.67	5	566	1755	5	30	0.92	9.50	4.30	105.00	4.20	714.00	61.80	24.00	10.30	17.00	31.60	0.907	-1.3	-0.2	0.732	-1.1	0.0	0.441	-2.6	-1.6
28	b	Jayanthi	691271F	daughter	33		157	60	24.34	3	562	12987	4	200	0.80	9.00	3.80	61.00	4.20	536.50	63.00	20.20	8.64	27.70	44.00	0.980	-0.6	-0.6	0.678	-1.5	-1.4	0.515	-0.9	-0.7
29	a	Pownammal	692540F	mother	52	40	145	53	25.21	4	650	4386	5	420	0.82	8.90	4.70	92.00	4.30	819.60	71.40	34.90	8.44	19.00	78.50	0.718	-3.0	-2.1	0.589	-2.3	-1.4	0.503	-1.4	-0.6
29	b	Sumathi	692543F	daughter	32		155	56	23.31	2	640	4386	5	120	0.76	9.20	4.00	60.00	4.90	523.10	60.50	22.90	9.88	22.00	58.50	0.987	-0.5	-0.5	0.860	0.1	0.2	0.560	-0.3	-0.2
30	a	Vijaya	692546F	mother	53	41	148	62	28.31	1	560	6343	5	10	1.04	9.50	5.30	80.00	4.80	610.00	8.10	9.10	9.67	23.80	24.40	1.257	1.9	2.9	0.913	0.6	1.5	0.620	0.8	1.6
30	b	Valarmathi	692549F	daughter	32		150	55	24.44	2	610	3739	4	120	0.89	8.80	3.90	76.00	4.30	676.90	49.30	18.10	11.40	12.65	66.60	0.832	-2.0	-1.9	0.776	-0.7	-0.5	0.635	1.0	1.2
31	a	Kasthuri	694373F	mother	50	49	152	44	19.04	2	890	3258	5	10	0.92	9.80	3.70	124.00	4.60	1,296.00	93.00	39.70	11.80	24.30	24.00	0.946	-0.9	-0.1	0.776	-0.7	0.1	0.530	-0.9	-0.2
31	b	Malarkodi	694378f	daughter	30		156	51	20.96	2	540	2970	3	90	0.85	9.80	3.50	86.00	4.70	479.50	46.70	22.10	12.91	19.30	40.60	0.919	-1.2	-1.2	0.699	-1.3	-1.3	0.565	-0.3	-0.2
32	a	Mayilammal	694368F	mother	55	49	157	35	14.20	4	500	810	5	240	0.76	8.90	3.20	117.00	3.90	260.20	42.90	16.20	6.20	30.20	34.80	0.529	-4.7	-3.6	0.485	-3.3	-2.2	0.355	-4.2	-3.2
32	b	Sumathi	694363F	daughter	33		157	40	16.23	2	480	6450	4	360	0.74	9.50	4.10	85.00	4.80	442.60	32.80	13.90	10.49	17.00	65.20	0.916	-1.2	-1.1	0.692	-1.4	-1.3	0.598	0.4	0.5
33	a	Lalitha	694353F	mother	65	45	147	44	20.36	3	490	279	5	15	0.78	9.60	3.90	99.00	4.70	518.80	55.90	18.80	9.79	15.80	51.90	0.626	-3.8	-2.0	0.617	-2.1	-0.5	0.428	-2.8	-1.2
33	b	Maragatham	694359F	daughter	49		157	70	28.40	2	630	4263	4	30	0.81	9.20	3.60	93.00	4.40	296.70	58.70	14.50	9.59	25.60	47.40	1.247	1.8	2.5	1.032	1.7	2.3	0.635	1.0	1.7
34	a	Mageshwari	605946D	mother	53	53	149	63	28.38	0	500	939	5	10	1.08	9.70	4.20	109.00	4.50	406.80	64.70	21.80	10.20	15.30	56.90	1.089	0.4	1.4	0.902	0.5	1.5	0.611	0.6	1.5
34	b	Vishalakshi	694346F	daughter	30		149	45	20.27	6	510	4360	4	30	0.75	9.40	3.10	82.00	4.50	286.30	46.00	18.90	9.00	13.20	43.30	0.921	-1.1	-1.1	0.794	-0.5	-0.4	0.552	-0.5	-0.4
35	a	Maniamma	987062A	mother	64	40	156	61	25.07	6	510	1260	4	10	0.90	9.50	4.10	111.00	4.40	471.80	60.90	31.60	10.00	51.40	58.60	0.673	-3.4	-1.7	0.577	-2.5	-1.0	0.407	-3.2	-1.6
35	b	Prema	694348F	daughter	43		160	59	23.05	4	540	4458	5	120	0.76	9.00	3.90	80.00	4.10	480.40	37.50	14.80	7.90	22.70	87.40	1.041	-0.1	0.3	0.853	0.0	0.4	0.505	-1.4	-0.9
36	a	Vendammal	697328F	mother	60	45	151	60	26.31	3	520	2746	5	360	0.63	9.00	4.20	90.00	4.00	1,321.00	104.00	38.60	18.80	18.95	39.80	0.664	-3.5	-2.0	0.609	-2.2	-0.8	0.334	-4.5	-3.3
36	b	Sasirekha	697327F	daughter	38		155	64	26.64	2	670	2747	4	120	0.95	8.80	4.10	57.00	4.80	753.40	96.30	31.20	14.88	21.00	26.80	0.943	-0.9	-0.8	0.703	-1.3	-1.1	0.531	-0.9	-0.6
37	a	Unnamalai	697331F	mother	70	42	149	49	22.07	3	740	9366	5	240	0.74	9.20	4.70	79.00	4.50	800.30	49.20	21.60	13.03	21.70	39.10	0.722	-3.0	-0.8	0.635	-1.9	-0.1	0.421	-2.9	-0.9
37	b	Amutha	697329F	daughter	35		153	44	18.80	2	600	10550	4	30	0.71	9.00	3.70	61.00	4.40	610.40	54.70	26.40	12.90	27.00	48.80	0.709	-3.1	-3.0	0.621	-2.1	-1.9	0.482	-1.8	-1.6
38	a	Suguna	697334F	mother	62	51	146	75	35.18	2	600	1470	5	20	0.94	9.50	4.10	94.00	4.20	370.20	31.70	15.60	9.63	23.70	56.80	0.794	-2.3	-0.7	0.834	-0.1	1.3	0.465	-2.1	-0.7
38	b	Savuri	697332F	daughter	40		147	65	30.08	3	690	3711	5	60	0.83	9.10	4.00	83.00	4.30	375.90	51.20	18.70	9.86	20.80	48.30	0.803	-2.2	-2.0	0.791	-0.5	-0.2	0.556	-0.4	-0.1
39	a	Baby	697337F	mother	50	48	152	55	23.81	2	710	3015	5	20	0.67	9.30	3.60	135.00	4.20	474.50	25.00	15.30	13.63	13.60	56.80	0.673	-3.4	-2.6	0.607	-2.2	-1.4	0.398	-3.4	-2.6
39	b	Rosy	697324F	daughter	30		157	74	30.02	1	740	2130	4	60	0.83	9.70	3.20	84.00	4.70	413.90	37.60	14.40	8.64	14.60	28.90	0.944	-0.9	-0.9	0.825	-0.2	-0.1	0.398	-3.4	-2.6

sno	id	name	cmch	relation	age	meno_age	ht	wt	BMI	PARITY	Diet_Cal	Phy_activity	SES	sun light	creat	calcium	phosph	ALP	albumin	S.CTX	S.P1NP	S.OC	Ur.DPD	Vit.D	PTH	LS_BMD	LS_T	LS_Z	LT_FN_BMD	LT_FN_T	LT_FN_Z	FA_BMD	FA_Ts	FA_Zc
40	a	Jamuna	697336F	mother	51	44	151	68	29.82	3	850	3276	4	15	0.76	9.40	5.00	99.00	4.30	584.00	49.30	16.10	11.04	6.24	42.80	1.041	-0.1	0.8	0.952	0.9	1.8	0.546	-0.6	0.2
40	b	Subashini	697340F	daughter	30		155	67	27.89	1	630	3706	3	45	0.72	9.50	3.60	72.00	4.40	271.40	42.30	15.40	8.60	29.90	37.30	1.041	-0.1	0.0	0.897	0.4	0.5	0.545	-0.6	-0.5
41	a	Rajathi	697830F	mother	55	40	145	49	23.31	3	700	1631	5	180	0.75	9.30	4.20	176.00	4.50	845.80	100.80	34.60	12.66	13.50	50.00	0.772	-2.5	-1.4	0.545	-2.7	-1.6	0.407	-3.2	-2.2
41	b	Nagammal	697835F	daughter	43		140	42	21.43	2	600	2277	4	420	0.85	9.00	3.30	80.00	4.70	353.00	53.80	14.70	6.50	28.70	31.40	1.014	-0.3	0.1	0.749	-0.9	-0.5	0.517	-1.2	-0.7
42	a	Kamala	697849F	mother	55	45	150	49	21.78	3	700	1854	5	360	0.92	9.30	4.00	100.00	4.20	808.50	90.30	35.50	11.69	39.20	20.90	0.632	-3.8	-2.7	0.519	-3.0	-1.9	0.372	-3.8	-2.9
42	b	Kala	697851F	daughter	30		160	65	25.39	5	580	1260	5	60	1.09	9.70	4.60	89.00	4.60	917.20	147.80	47.00	9.22	26.00	21.50	0.875	-1.0	-1.0	0.756	-0.8	-0.7	0.545	-0.6	-0.5
43	a	Kala	697839F	mother	54	52	145	57	27.11	2	680	5775	5	20	0.95	9.00	3.80	86.00	4.60	297.70	42.70	15.50	10.52	13.20	71.80	0.994	-0.5	0.6	0.749	-0.9	0.1	0.535	-0.8	0.1
43	b	Kumari	697842F	daughter	38		154	62	26.14	2	560	2274	4	30	0.89	8.90	3.70	68.00	4.70	160.80	24.70	12.20	11.40	17.80	41.60	1.111	0.6	0.8	0.862	0.1	0.4	0.577	0.0	0.3
44	a	Kasthuri	167160c	mother	71	45	143	48	23.47	4	380	739	5	10	1.19	9.00	3.50	70.00	4.60	447.70	31.50	22.10	9.30	33.80	72.60	0.703	-3.1	-0.9	0.572	-2.5	-0.6	0.404	-3.2	-1.1
44	b	Tamarai Selvi	699529F	daughter	45		138	38	19.95	2	500	4850	5	15	0.78	8.80	3.60	56.00	4.30	300.00	23.50	16.80	5.24	15.00	50.40	0.928	-1.1	-0.6	0.764	-0.8	-0.3	0.551	-0.5	0.0
45	a	Chandra	177022C	mother	73	42	154	54	22.77	2	520	2088	5	30	0.97	9.20	3.90	88.00	4.50	423.00	49.80	23.10	7.80	20.84	32.80	0.665	-3.5	-1.1	0.604	-2.2	-0.2	0.467	-2.1	0.3
45	b	Latha	699531F	daughter	47		156	66	27.12	5	460	5832	4	20	0.78	8.90	3.80	73.00	4.30	240.10	39.20	16.90	8.98	15.80	38.10	1.036	-0.1	0.5	0.864	0.1	0.7	0.578	0.0	0.6
46	a	Baby	699523F	mother	65	45	150	56	24.89	7	400	693	2	10	0.90	8.90	4.30	87.00	3.70	552.60	55.30	19.70	14.70	14.60	35.30	0.717	-3.0	-1.2	0.579	-2.4	-0.9	0.392	-3.5	-1.8
46	b	Tharageswari	699524F	daughter	38		143	60	29.34	2	800	3200	4	15	0.92	8.50	3.80	69.00	4.30	550.60	51.50	20.40	8.60	17.82	43.50	0.945	-0.9	-0.8	0.774	-0.7	-0.4	0.616	0.7	1.0
47	a	Banumathi	701091f	mother	55	43	153	74	31.61	3	750	3199	4	120	1.14	10.10	3.90	118.00	4.10	720.60	80.30	31.30	8.06	20.20	38.40	0.727	-2.9	-1.8	0.738	-1.0	0.1	0.464	-2.1	-1.1
47	b	Sumathi	701092F	daughter	32		153	68	29.05	1	740	10950	4	300	0.91	10.00	4.00	71.00	4.40	149.60	39.10	17.30	11.05	16.00	42.80	0.817	-2.1	-2.1	0.723	-1.1	-1.0	0.591	0.2	0.4
48	a	Vijaya	701094F	mother	55	45	157	49	19.88	3	700	2781	5	180	0.93	9.90	3.70	97.00	4.30	992.30	57.90	33.00	12.30	27.20	52.10	0.752	-2.7	-1.6	0.720	-1.2	-0.1	0.439	-2.6	-1.6
48	b	Rani	701089F	daughter	33		154	62	26.14	3	550	4896	4	180	0.87	9.50	3.40	80.00	4.20	638.30	42.90	23.00	10.65	21.80	58.50	0.915	-1.2	-1.2	0.770	-0.7	-0.6	0.498	-1.5	-1.3
49	a	Kasthuri	700881F	mother	65	45	143	50	24.45	5	490	513	5	15	0.76	9.60	3.80	104.00	4.20	631.00	60.60	21.70	11.50	27.80	33.90	0.650	-3.6	-1.8	0.642	-1.9	-0.3	0.433	-2.7	-1.1
49	b	Usha	700877F	daughter	44		150	44	19.56	3	510	4435	4	60	0.85	9.40	3.10	68.00	4.20	336.90	31.70	13.90	8.58	24.20	41.20	0.872	-1.6	-1.2	0.760	-0.8	-0.4	0.578	0.0	0.5
50	a	Rani	700871F	mother	65	40	155	55	22.89	3	590	3255	5	180	0.87	10.70	3.90	117.00	4.70	679.50	70.00	31.30	12.97	28.80	34.00	0.775	-2.5	-0.7	0.653	-1.8	-0.2	0.517	-1.1	0.5
50	b	Kalpana	700875F	daughter	42		157	73	29.62	2	690	12228	5	240	0.75	9.80	3.50	71.00	4.30	302.40	36.00	14.40	10.14	18.65	29.90	1.109	0.6	0.9	0.831	-0.2	0.2	0.601	0.4	0.8
51	a	Panjalai	700865F	mother	55	51	151	72	31.58	3	540	1150	4	90	0.86	10.20	3.70	85.00	4.80	595.60	59.00	31.70	13.01	18.60	56.90	0.966	-0.7	0.4	0.819	-0.3	0.8	0.612	0.6	1.6
51	b	Geetha	700866F	daughter	30		153	73	31.18	0	680	1815	4	15	0.84	9.90	3.10	71.00	4.70	300.60	40.50	16.00	11.20	10.90	27.20	1.272	2.0	2.1	0.887	0.3	0.4	0.710	2.4	2.6
52	a	Rajeswari	700864F	mother	71	50	160	80	31.25	1	690	2682	4	30	0.89	9.50	3.50	110.00	4.60	623.60	70.90	36.90	14.90	5.80	63.10	0.801	-2.2	0.0	0.580	-2.4	-0.5	0.538	-0.8	1.4
52	b	Revathi	700859F	daughter	40		168	77	27.28	3	730	2166	5	5	0.88	9.90	3.70	79.00	4.40	487.50	87.30	24.50	8.20	20.70	38.00	0.913	-1.2	-1.0	0.784	-0.6	-0.3	0.532	-0.9	-0.5

sno	id	name	cmch	relation	age	meno_age	ht	wt	BMI	PARITY	Diet_Cal	Phy_activity	SES	sun light	creat	calcium	phosph	ALP	albumin	S.CTX	S.P1NP	S.OC	Ur.DPD	Vit.D	PTH	LS_BMD	LS_T	LS_Z	LT_FN_BMD	LT_FN_T	LT_FN_Z	FA_BMD	FA_Ts	FA_Zc
53	a	Selvarani	700856f	mother	55	50	151	47	20.61	4	690	13670	4	240	1.14	9.90	3.60	98.00	4.30	806.30	81.10	29.60	4.50	40.60	68.30	0.780	-2.4	-1.3	0.620	-2.1	-1.0	0.480	-1.8	-0.9
53	b	Mercy Volena	700854F	daughter	29		153	59	25.20	2	560	5022	3	20	0.85	9.20	3.90	87.00	4.50	863.10	65.30	18.40	7.90	23.50	67.60	1.021	-0.2	-0.2	0.713	-1.2	-1.2	0.535	-0.8	-0.7
54	a	Saroja	981877D	mother	55	42	152	64	27.70	3	580	3402	4	120	0.82	9.90	4.60	66.00	4.60	558.30	45.30	20.00	16.80	25.30	32.30	0.806	-2.2	-1.1	0.793	-0.5	0.6	0.524	-1.0	-0.1
54	b	Sangeetha	646303B	daughter	32		148	60	27.39	3	460	4428	1	180	0.78	9.80	3.30	79.00	4.70	206.00	28.10	11.70	11.80	24.70	50.80	0.895	-1.4	-1.3	0.744	-0.9	-0.8	0.530	-0.9	-0.7
55	a	Amsa	706906F	mother	55	50	150	58	25.78	2	650	495	3	60	0.65	8.90	4.20	121.00	4.50	861.50	100.50	30.90	11.57	24.90	62.00	0.578	-4.3	-3.1	0.584	-2.4	-1.3	0.480	-1.8	-0.9
55	b	Tamilselvi	706908F	daughter	30		150	62	27.56	2	630	1350	4	30	0.75	8.90	3.40	88.00	4.80	249.00	52.80	20.30	8.09	15.50	45.90	0.928	-1.1	-1.1	0.805	-0.4	-0.3	0.544	-0.6	-0.5
56	a	Sembai	706902F	mother	55	40	155	40	16.65	3	560	12867	5	240	0.66	8.90	4.20	104.00	4.60	1,025.00	74.40	50.30	9.26	28.40	83.60	0.587	-4.2	-3.1	0.651	-1.8	-0.7	0.329	-4.6	-3.6
56	b	Dhanalakshmi	706904F	daughter	32		150	64	28.44	2	640	1557	4	30	0.74	8.50	3.60	62.00	4.40	552.70	42.10	20.00	11.90	25.80	82.00	1.082	0.3	0.4	1.075	2.0	2.2	0.520	-1.1	-0.9
57	a	Pushpa	706920F	mother	55	50	155	85	35.38	3	600	537	5	30	0.73	8.80	3.50	102.00	4.30	536.50	59.10	23.20	14.45	20.00	75.00	0.822	-2.0	-0.9	0.700	-1.3	-0.3	0.546	-0.6	0.4
57	b	Lavanya	706921F	daughter	30		150	55	24.44	2	640	2730	3	180	0.71	8.70	3.70	73.00	4.40	257.20	38.40	14.70	7.44	17.00	51.30	1.017	-0.3	-0.3	0.812	-0.3	-0.2	0.595	0.3	0.4
58	a	Rani	706925F	mother	60	50	160	58	22.66	4	730	3909	5	60	0.78	9.10	4.60	97.00	4.30	379.70	81.40	38.00	9.72	22.00	47.70	0.624	-3.8	-2.4	0.602	-2.2	-0.9	0.626	0.9	1.0
58	b	Mahalakshmi	706919F	daughter	30		170	72	24.91	0	770	1989	4	20	0.80	9.30	3.70	79.00	5.00	291.80	60.60	17.10	12.44	18.00	40.80	0.950	-0.9	-0.9	0.694	-1.4	-1.3	0.403	-3.3	-2.0
59	a	Govindammal	707664F	mother	55	51	156	49	20.13	4	510	1040	5	30	0.81	10.70	3.70	72.00	5.00	465.80	58.40	33.70	12.09	39.70	30.20	0.810	-2.2	-1.0	0.759	-0.8	0.3	0.421	-2.9	-2.0
59	b	Navaneetham	707663F	daughter	34		156	69	28.35	2	690	3570	5	20	0.83	10.90	4.60	52.00	5.00	188.00	37.80	14.80	11.90	15.00	24.50	1.259	1.9	2.0	0.912	0.6	0.7	0.523	-1.0	-0.8
60	a	Malliga	707661F	mother	55	52	161	56	21.60	2	600	7629	5	180	0.75	10.10	4.20	107.00	4.20	760.00	71.40	25.30	11.03	21.50	43.10	0.887	-1.5	-0.3	0.707	-1.3	-0.2	0.474	-1.9	-1.0
60	b	Vasanthi	707659F	daughter	32		163	64	24.09	3	600	12318	4	360	0.93	10.30	3.90	96.00	4.50	209.80	33.50	11.90	5.70	16.00	39.10	0.938	-1.0	-1.0	0.830	-0.2	0.0	0.626	0.9	1.0
61	a	Indhirani	707667F	mother	60	49	152	41	17.75	4	600	4437	4	360	0.73	9.80	4.90	92.00	4.40	1,360.00	147.80	65.80	15.40	36.00	17.80	0.681	-3.3	-1.9	0.570	-2.5	-1.2	0.326	-4.7	-3.4
61	b	Arputham	707659f	daughter	40		155	57	23.73	4	560	10365	4	240	0.85	9.90	3.40	86.00	4.30	484.20	66.90	19.90	9.27	16.20	19.40	0.947	-0.9	-0.7	0.660	-1.7	-1.4	0.469	-2.0	-1.7
62	a	Saraswathi	708381F	mother	55	53	162	72	27.43	2	510	1625	4	360	0.71	8.90	3.50	154.00	3.80	650.00	46.20	20.80	10.53	6.04	66.00	0.887	-1.5	-0.3	0.646	-1.8	-0.7	0.517	-1.1	-0.2
62	b	Ananthi	708376F	daughter	30		146	60	28.15	2	600	2846	4	120	0.83	9.20	3.30	46.00	4.60	372.00	37.40	18.60	3.97	26.00	51.30	1.068	0.2	0.2	0.842	-0.1	0.0	0.601	0.4	0.5
63	a	Deivanai	708388F	mother	60	50	148	52	23.74	3	550	9519	5	360	0.80	9.40	4.20	148.00	4.30	436.10	58.90	23.80	7.48	36.10	32.90	0.726	-2.9	-1.4	0.647	-1.8	-0.5	0.562	-0.3	1.0
63	b	Suganthi	708395F	daughter	30		154	50	21.08	3	800	1260	4	15	0.76	9.80	4.00	46.00	4.90	254.90	69.40	21.70	11.18	18.00	40.10	0.950	-0.9	-0.9	0.860	0.1	0.2	0.622	0.8	0.9
64	a	Ganapathiamma	708397F	mother	60	55	145	55	26.16	3	550	5430	5	360	0.79	9.00	3.40	102.00	4.10	814.10	59.30	24.00	11.05	15.90	53.70	0.983	-0.6	0.9	0.713	-1.2	0.1	0.553	-0.5	0.8
64	b	Shanthi	708391F	daughter	30		156	52	21.37	1	600	2867	4	300	0.81	9.20	3.30	69.00	4.30	604.60	76.30	23.10	9.97	25.90	35.40	1.010	-0.3	-0.3	0.717	-1.2	-1.1	0.580	0.0	0.1
65	a	Saroja	709105F	mother	60	40	150	63	28.00	5	540	1680	3	180	0.84	9.10	4.50	57.00	4.50	216.00	47.40	14.30	10.74	20.10	47.70	0.992	-0.5	1.0	0.706	-1.3	0.0	0.402	-3.3	-2.0
65	b	Manjula	709113F	daughter	36		155	75	31.22	1	540	2100	4	10	0.96	9.20	3.40	68.00	12.50	437.20	67.30	31.60	9.62	20.20	43.20	1.045	0.0	0.1	0.908	0.5	0.7	0.552	-0.5	-0.3

sno	id	name	cmch	relation	age	meno_age	ht	wt	BMI	PARITY	Diet_Cal	Phy_activity	SES	sun light	creat	calcium	phosph	ALP	albumin	S.CTX	S.P1NP	S.OC	Ur.DPD	Vit.D	PTH	LS_BMD	LS_T	LS_Z	LT_FN_BMD	LT_FN_T	LT_FN_Z	FA_BMD	FA_Ts	FA_Zc
66	b	Jeyanthi	709124F	daughter	31		164	44	16.36	3	600	13503	4	360	0.88	8.90	5.00	76.00	4.60	581.00	49.70	22.10	8.14	15.00	49.00	1.041	-0.1	0.0	0.771	-0.7	-0.6	0.580	0.0	0.2
67	a	Santha	709129F	mother	65	45	160	68	26.56	3	500	630	5	30	0.71	9.00	3.70	141.00	4.50	749.70	54.10	20.70	9.05	20.40	48.40	0.712	-3.0	-1.2	0.596	-2.3	-0.7	0.400	-3.3	-1.7
67	b	Neelaveni	709125F	daughter	45		151	75	32.89	4	650	7628	5	300	0.85	8.80	3.50	119.00	4.10	541.70	50.60	24.90	9.20	20.80	50.60	0.916	-1.2	-0.7	0.804	-0.4	0.1	0.601	0.4	0.9
68	a	Chinna Kannu	710343F	mother	65	45	158	59	23.63	4	560	729	5	15	0.92	9.30	4.70	67.00	4.40	613.00	61.90	39.70	12.36	29.40	22.00	0.827	-2.0	-0.2	0.740	-1.0	0.6	0.400	-3.3	-1.7
68	b	Alamelu	710345F	daughter	46		156	56	23.01	3	640	2913	4	300	0.92	8.70	4.00	52.00	4.30	198.60	31.30	16.90	8.46	22.90	42.20	1.156	1.0	1.5	0.751	-0.9	-0.4	0.552	-0.3	0.3
69	a	Saradha	710348f	mother	65	40	140	44	22.45	5	540	1701	5	60	0.75	10.00	4.00	90.00	4.40	494.90	42.60	27.80	11.20	37.00	42.20	0.640	-3.7	-1.9	0.560	-2.6	-1.0	0.270	-5.7	-4.1
69	b	Sivasakthi	710349F	daughter	40		157	57	23.12	1	560	3696	5	60	0.66	9.80	3.60	122.00	4.50	848.90	76.90	33.80	11.67	18.40	80.60	0.681	-3.3	-3.1	0.621	-2.1	-1.7	0.548	-2.4	-2.1
70	a	Janaki	710358F	mother	53	40	151	62	27.19	3	580	4335	4	180	0.83	10.10	3.70	104.00	4.30	925.50	48.00	20.80	9.70	21.20	49.00	0.725	-2.9	-1.9	0.597	-2.3	-1.3	0.465	-2.1	-1.2
70	b	Kavitha	710351F	daughter	25		150	69	30.67	4	540	2100	4	20	0.81	10.10	3.70	71.00	4.40	140.10	33.30	16.20	6.98	16.22	42.90	1.128	0.7	0.8	0.858	0.1	0.1	0.598	0.2	0.2
71	a	Gowri	710364F	mother	60	45	155	48	19.98	1	550	3564	5	240	0.81	10.20	3.70	86.00	4.40	476.40	42.60	25.00	7.80	21.30	41.00	0.747	-2.7	-1.3	0.617	-2.1	-0.8	0.399	-3.3	-2.1
71	b	Maragatham	710360F	daughter	33		152	60	25.97	2	540	2940	4	120	0.70	10.00	3.20	83.00	4.40	189.80	33.90	12.70	7.98	26.00	26.50	1.027	-0.2	-0.1	0.669	-1.6	-1.5	0.525	-1.0	-0.8
72	a	Ambiga	711524F	mother	61	51	153	63	26.91	4	680	2340	5	120	1.01	9.10	4.80	108.00	4.50	454.10	56.60	22.70	8.22	17.10	41.60	1.033	-0.1	1.4	0.732	-1.1	0.3	0.541	-0.7	0.7
72	b	Vanitha	711519F	daughter	30		154	53	22.35	3	600	2804	4	120	0.96	9.70	4.90	71.00	4.80	84.60	20.10	7.60	4.85	21.20	22.50	1.073	0.2	0.2	0.840	-0.1	0.0	0.568	-0.2	-0.1
73	a	Hamsaveni	711517F	mother	60	50	157	43	17.44	7	650	8016	5	240	0.94	9.40	3.60	64.00	4.40	734.40	64.50	31.40	9.02	15.10	31.20	0.651	-3.6	-2.1	0.619	-2.1	-0.8	0.437	-2.6	-1.3
73	b	Valarmathi	711513F	daughter	40		155	49	20.40	4	650	2237	4	300	0.81	9.80	3.30	47.00	4.70	184.50	36.20	15.40	7.50	16.00	24.00	1.042	0.0	0.2	0.813	-0.3	0.0	0.590	0.2	0.5
74	a	Gowri	711526F	mother	60	40	148	51	23.28	8	650	984	5	240	0.68	10.20	3.20	117.00	4.50	768.60	61.00	20.72	11.80	16.70	120.60	0.902	-1.3	0.2	0.680	-1.5	-0.2	0.459	-2.2	-0.9
74	b	Eswari	711529F	daughter	40		155	55	22.89	3	610	3318	4	60	0.90	9.30	2.40	70.00	4.30	344.40	42.40	16.10	9.65	14.10	94.50	1.116	0.6	0.9	0.814	-0.3	0.0	0.530	-0.9	-0.6
75	a	Selvarani	711541F	mother	55	40	148	55	25.11	4	630	4305	5	240	0.73	9.50	3.50	98.00	4.60	346.40	41.50	18.20	9.15	32.70	76.70	0.852	-1.8	-0.6	0.639	-1.9	-0.8	0.493	-1.6	-0.6
75	b	Saridha	711537F	daughter	30		147	53	24.53	2	640	3006	4	30	0.86	9.90	3.50	77.00	4.70	576.70	47.80	23.40	9.59	15.10	46.50	1.059	0.1	0.1	3.430	0.8	-0.8	6.320	0.6	-0.4
76	a	Saroja	727869F	mother	55	45	148	46	21.00	3	630	5490	4	360	0.86	8.90	4.00	62.00	4.80	452.80	52.50	17.10	13.10	30.10	38.20	0.902	-1.3	-0.7	0.635	-1.9	-0.8	0.480	-1.8	-0.9
76	b	sudha	727871F	daughter	30		152	56	24.24	2	600	2250	4	360	0.78	8.60	2.70	65.00	4.60	479.30	30.00	14.60	8.80	20.30	91.80	1.113	0.6	0.6	0.861	0.1	0.2	0.557	-0.4	-0.3